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CARBON DIOXIDE FROM THE NERVE CORD OF THE LOBSTER.

By G. H. PARKER.

(From the Zoological Laboratory, Harvard University, Cambridge.)

(Accepted for publication, April 14, 1925.)

I. INTRODUCTION.

Since it has been shown in a previous paper (Parker, 1925) that CO_2 is given out in measurable quantities by the lateral-line nerve of the dogfish and that this gas increases in amount when the nerve from which it comes is stimulated, it is natural to inquire if like occurrences take place in other types of nervous tissue. The lateral-line nerve is a purely sensory nerve and the pieces used in the tests just referred to were entirely devoid of ganglion cells. This nerve therefore represented a transmitting organ uncomplicated by any central connections or activities. In the present study a portion of the central nervous system, the nerve cord of the lobster (*Homarus americanus* Milne-Edwards), has been selected because this part includes not only nerve fibers but also ganglion cells and fibrillar material, all the components of a complicated central structure. The portion of the nerve cord chosen was the section including the last three abdominal ganglia. This section could be easily dissected out as a single strand and could be handled with convenience in experimentation.

The apparatus used in this study and the general technique employed have been described in the paper, already referred to, on the lateral-line nerve. In the present study as in the previous one the rates of CO_2 production have been expressed in milligrams of CO_2 per gram of nervous material per minute. This form of expression allows easy comparisons with the results of earlier work. Quantitative studies on the CO_2 from ganglionated organs have already been reported by Tashiro and Adams (1914) and by Moore (1922).

II. OBSERVATIONS.

The nerve cord of the lobster is extremely delicate as compared with the lateral-line nerve of the dogfish. Tests quickly demonstrated that after its removal from the animal it could not be relied upon as a satisfactory preparation for much more than two hours. Hence all results reported in this paper are based upon determinations made within an hour or so of the time when the cord was excised. The relatively rapid decline in the vitality of the cord of the lobster is apparently duplicated by that of the crawfish where Moore (1922) found it well to restrict his period of observation to the first half hour after dissection. This condition is in strong contrast to that of nerves from cold-blooded vertebrates which may be successfully worked upon for hours after their removal from the animal.

When the last three ganglia of the nerve cord of the lobster are put in a closed type of respiratory chamber and tested for CO_2, the discharge of this gas can be shown to be relatively high at the outset and to fall off rapidly till by the end of an hour it becomes fairly low. The change of rate seen in this fall is well illustrated in Fig. 1 where are plotted the rates of CO_2 discharge from three preparations of the ventral cord. In cord I the rate was at the beginning 0.2330 mg. CO_2 per gram of cord per minute. It then fell successively to 0.1882, 0.1378 and finally to 0.0915 mg. Cord II began at 0.1858 mg. and ended at 0.0619 mg., and cord III at 0.1678 mg. and ended at 0.0618 mg. An inspection of the plottings in Fig. 1 shows that it is not possible to divide the CO_2 production of the lobster cord into the two periods, one of initial gush and the other of steady outflow, as could be done with the lateral-line nerve of the dogfish (Parker, 1925). In the lobster cord the discharge of CO_2 is on a steadily decreasing rate which never passes over into a period of uniformity as with dogfish nerve. The process resembles a prolonged gush which reaches from beginning to end. At the outset of the discharge the average rate for the three cords tested was 0.19553 mg. CO_2 per gram of cord per minute and at the end 0.07173 mg., a falling off to nearly one-third the original rate. In consequence of this condition it is impossible to give any average rate that may be taken to represent the rate of a quiescent cord, for such a quiescent condition does not exist in the prepared cord. All that can be stated is that during the first hour after the

preparation of the cord the average rate of output of CO_2 in round figures declines from 0.20 mg. at the beginning to 0.07 mg. at the end. Taking all in all this is a higher rate than that shown by the lateral-line nerve of the dogfish which averaged 0.0095 mg. (Parker, 1925).

Tashiro and Adams (1914) have measured by means of the biometer the rate of CO_2 production of a piece of nerve and of the heart ganglion of *Limulus* and have found the nerve to discharge 2.6×10^{-7} and the

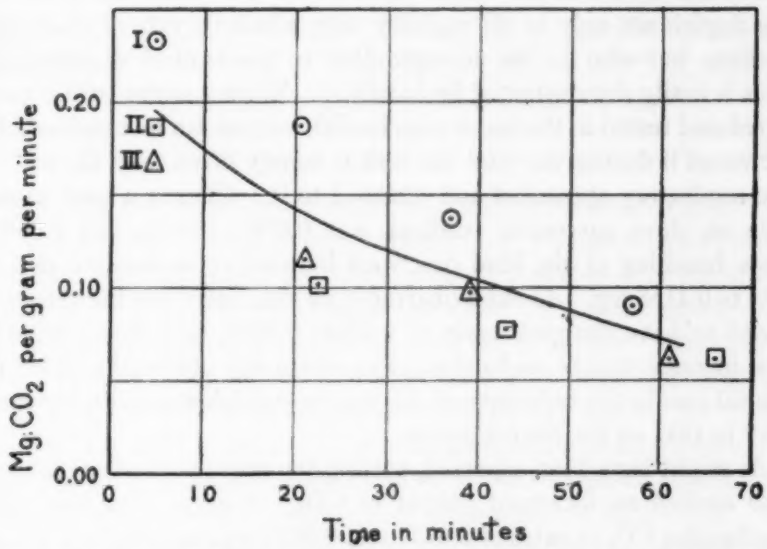

FIG. 1. Plottings of the respiratory rates in milligrams of CO_2 per gram of cord per minute for three nerve-cord preparations from the lobster, *Homarus americanus*. The readings from Cord I are represented by dots inclosed within circles; from Cord II by dots within squares; and from Cord III by dots within triangles. Temperature about 23°C.

ganglion 2.3 to 4.7×10^{-7} grams CO_2 per centigram of tissue per 10 minutes. Transposing these determinations into the form of expression used in this paper, the ganglion may be said to discharge from 0.0023 to 0.0047 mg. CO_2 per gram of ganglion per minute. These rates are about one-thirtieth of those which I have found for the lobster cord. The occasion of this discrepancy is not easily discovered. I do not believe it to be due to a real difference in the nature of the two tissues tested. It is in my opinion rather a matter of technique. As

I have already observed (Parker, 1925), the biometer method of Tashiro though very sensitive from a qualitative standpoint, is probably not reliable for quantitative work. At least this is my experience with it and in this my results agree with those of Adam (1921). I therefore believe that the determinations of Tashiro and Adams just quoted are less likely to prove accurate than those given in this paper.

The nerve cord of the lobster differs from the lateral-line nerve of the dogfish not only in the rapidity with which its rate of discharge declines but also in its susceptibility to mechanical disturbance. This is easily demonstrated by handling. When a nerve cord is prepared and tested in the usual way, its CO_2 output can be considerably increased if during the trial the cord is merely lifted from the rod of the respiratory apparatus and returned to it. Thus in a cord whose rate on three successive readings was 0.0793, 0.0705, and 0.0693, mere handling of the kind described induced an immediate rise of rate to 0.1168 mg. About a quarter of an hour after this the rate was found to have dropped again to 0.0764, 0.0681, and finally 0.0644. The increase of rate on handling was repeatedly observed and led to special care in the technique of this research which was quite unnecessary in that on the dogfish nerve.

As might have been expected, cutting the nerve cord of the lobster also excited an increased output of CO_2. A nerve cord that was discharging CO_2 at rates of 0.0727 and 0.0670 mg. on being cut across in eight places showed immediately an increased rate of 0.1049 mg. which quickly subsided, however, to 0.0954, 0.0795, and finally 0.0700 mg. Thus both handling and cutting strikingly increase for a brief period the CO_2 discharged from the nerve cord of the lobster and indicate its relatively high sensitiveness to mechanical stimulation. This delicacy is quite in line with what is known of such nerve cord preparations. Only with the greatest care can they be kept alive for the one or two hours necessary for their study.

Is the CO_2 output of the nerve cord of the lobster influenced by stimulation? Since this cord, unlike the lateral-line nerve of the dogfish, does not show a period of uniform discharge of CO_2, somewhat different methods of testing it for an increased CO_2 output on stimulation were resorted to than those used with the dogfish nerve. In

Table I are given the successive rates of two cords alternately stimulated and unstimulated.

TABLE I.

Rates of CO_2 Production in Milligrams of CO_2 per Gram of Cord per Minute of Two Nerve Cords from the Lobster, Homarus americanus, Alternately Stimulated and Unstimulated. Temperature about 23°C.

Cord No.	Condition of cord.						
	St.	Unst.	St.	Unst.	St.	Unst.	St.
1	0.0911	0.0762	0.0770	0.0609	0.0698	0.0473	
2		0.0668	0.0782	0.0470	0.0673	0.0532	0.0612

In cord 1 the records begin with the stimulated condition at 0.0911 mg. and after five tests end with a record from the unstimulated state at 0.0473 mg. In cord 2 the relations are reversed in that the series begins with an unstimulated state and ends with a stimulated one. In both series, as can be seen by inspecting the table, there is an almost steady decline in the rate in such a direction that overweight is given to the kind of rate, stimulated or unstimulated, with which a particular series begins. Hence in cord 1 the rates for the stimulated states must be too high in comparison with those for the unstimulated states, and consequently averages from this series alone would be misleading. In cord 2 on the other hand the rates for the unstimulated state are presumably too high and, though the averages from this cord alone would be as misleading as those from cord 1, the general averages from both cords together ought to yield results of an unbiased kind. Averaging all the records in Table I for the stimulated condition yields 0.07410 mg. and for the unstimulated state 0.05857 mg. The difference between these two amounts, 0.01553 mg. represents the increase of rate in consequence of stimulation, an increase of 26.5 per cent of that for the quiescent cord. This amount is roughly 1.6 times that of the percentage increase (15.8 per cent) in stimulated lateral-line nerve (Parker, 1925).

This increase of rate in the nerve cord must represent CO_2 from a strictly nervous source, for it is the excess CO_2 produced on stimula-

tion. Such cannot be said of the CO_2 given out by the quiescent cord. As Bayliss (1915, p. 379) pointed out for nerve, the CO_2 of the quiescent state may come from connective tissue, from nerve proper, or from both and, until the proportion of these two possible contributions is ascertained, the percentage increase on a strictly nervous basis cannot be calculated.

Tashiro and Adams (1914) and Moore (1922) report for the ganglionated organs studied by them no significant difference in the CO_2 output on stimulation. The heart ganglion of *Limulus*, according to Tashiro and Adams, gave out CO_2 at about the same rate as a nerve from that animal and showed no increase on stimulation. The resting nerve cord of *Cambarus*, according to Moore, discharged CO_2 at a rate which for comparison was called 100. On stimulation this fell to 89 per cent and on final quiescence to 86 per cent. So far as the results of Tashiro and Adams are concerned, I have already (Parker, 1925) stated the grounds for my belief that the biometer, though it may be very sensitive for qualitative determinations, is not reliable for quantitative work. These authors have in my opinion shown beyond a doubt that the heart ganglion of *Limulus* discharges CO_2, but I believe that the method which they have used is not one adapted to demonstrate a change of rate in this discharge in consequence of stimulation.

Moore, working with a method that gave relative, not absolute, results showed in the nerve cord of *Cambarus* a decline of rate such as that recorded in this paper but since he was unable to extend his observations beyond a series of three readings he failed to find the significance of the downward steps which when inspected in as long a series as that shown in nerve I (Fig. 1) demonstrate a real increase of CO_2 on stimulation. Hence I cannot agree with the negative results of Tashiro and Adams and of Moore, but I conclude on the basis of the observations recorded in this paper that stimulated central nervous organs do show an increased rate of CO_2 production. Bayliss (1924, p. 379), in commenting on the results of these workers, expressed his doubts on the conclusion that stimulated ganglionated masses do not discharge CO_2 at a higher rate than nerve fibers and this doubt is entirely justified by what is reported in the present contribution.

III. SUMMARY.

1. The nerve cord of the lobster (*Homarus americanus* Milne-Edwards) is very delicate and can be used as a living preparation for only a few hours after its removal from the animal.

2. During the first hour or so after removal it discharges CO_2 at a steadily decreasing rate beginning at about 0.20 mg. CO_2 per gram of cord per minute and ending at about 0.07 mg.

3. This discharge exhibits a steady decrease in rate and is not divisible into a period of gush and a period of uniform outflow as with the lateral-line nerve of the dogfish. It terminates in a very few hours with the complete death of the cord.

4. Both handling and cutting the cord temporarily increase the rate of CO_2 output.

5. The stimulated cord discharges CO_2 at a rate about 26 per cent higher than that of the quiescent cord, an increase of about 1.6 times that of the increase observed in the lateral-line nerve of the dogfish under similar circumstances.

LITERATURE.

Adam, N. K. 1921. Note on the Oxygen Consumption of Amphibian Muscle and Nerve. Biochem. J., **15,** 358.

Bayliss, W. M. 1915. Principles of General Physiology. London.

Bayliss, W. M. 1924. Principles of General Physiology. 4th edition, London.

Moore, A. R. 1922. The Respiratory Rate of the Nerve Cord of Cambarus clarkii. Proc. Soc. Exp. Biol. Med., **19,** 335.

Parker, G. H. 1925. The Production of Carbon Dioxide by Nerve. J. Gen. Physiol., **7,** 641.

Riggs, L. K. 1919. Action of Salts upon the Metabolism of Nerves. J. Biol. Chem., **39,** 385.

Tashiro, S. and H. S. Adams. 1914. Comparison of the Carbon-dioxide Output of Nerve Fibers and Ganglia in Limulus. J. Biol. Chem., **18,** 329.

THE INHIBITION OF CYPRIDINA LUMINESCENCE BY LIGHT.

By E. NEWTON HARVEY.

(From the Physiological Laboratory, Princeton University, Princeton, and the Nela Research Laboratories, Cleveland.)

(Accepted for publication, April 17, 1925.)

Since Allman (1862) reported that Ctenophores would exhibit no luminescence in daylight, a similar inhibition of luminescence in light has been described for *Noctiluca* (Massart, 1893), *Ceratium* (Zacharias, 1905), *Ptychodera* (Crozier), *Pelagia* (Heymans and Moore, 1923–24), *Renilla* (Parker, 1920), and some other forms.

Most luminous animals shun the light. We might therefore expect to find an adaptive mechanism in animals, especially in such marine forms as are stimulated to luminescence by the agitation of the waves, by which luminescence would be prevented in daylight, thus saving luminous material.

In the intact Ctenophores, Moore (1923–24) believes the principal action of light to be through local photoreceptor nerves whose stimulation by light converts a preluminous material, A, into D, a non-luminescent product. In the dark, mechanical stimulation of tactile receptors would have converted A to L, the luminescent material, and the Ctenophore would shine. As the luminous slime of Ctenophores, removed by dragging the meridional canals over filter paper (Moore, 1923–24) will also have its luminescence suppressed by light, as well as filtered extracts of the animal (Harvey, 1924–25) in which nerve connections are surely severed, we must conclude that light may also suppress luminescence by a direct effect on the luminous cells and cell fragments.

In the ostracod crustacean, *Cypridina*, a fluid is secreted from gland cells near the mouth. In contact with the dissolved oxygen of the sea water this fluid luminesces. It contains two substances, luciferin and luciferase, both necessary for luminescence. It should

be noted that, once projected into the sea water, the luminous fluid is never again recovered by the animal. We should therefore expect that if *Cypridina* is to conserve luminous material adaptively there should be an inhibitory effect of light on the secretory mechanism. I do not know whether light inhibits the secretion of luminous material or not, but I have recently noted a rapid inhibitory effect of light on the luminescent secretion itself. One wonders what the significance of this inhibition can be. There is no doubt that in this case light acts directly on the luminescent reaction with no possibility of nerve influence. Moreover, it is a simple matter to determine which of the two substances, luciferin or luciferase, is affected by illumination.

The experiments are carried out in the following manner. The light from a carbon arc (soft cored 13 mm. diameter carbons, at right angles, using 15 amperes at 55 volts = 825 watts), in a dark house, after passing through 60 mm. water, is condensed to a slightly converging beam by a lens 135 mm. in diameter. The beam passes through a black tube with a screen at the end containing a slit 8 mm. wide × 20 mm. long, so that all light is excluded from the dark room except a narrow band, 8 mm. × 20 mm., in whose path a small test-tube of luminescent solution may be placed. The illumination in the region of the test-tube is about 15,000 foot candles, much greater than sunlight at noon in summer (10,000 foot candles), but the light had passed through glass so that all deleterious ultra-violet rays were removed. A camera shutter for rapid screening of the beam was placed before the test-tube so that it could be examined very quickly after exposure. Since the beam is narrow (8 mm.) only a narrow area of the test-tube need be exposed to light, the portions above and below the beam remaining in comparative darkness. Thus we have the opportunity of examining two contiguous areas of luminescent solution, one of which has been illuminated and the other not, and any change in intensity of luminescence may be easily observed.

By mixing large amounts of *Cypridina* luciferin with a small amount of *Cypridina* luciferase, the resultant solution will emit a plainly visible continuous luminescence whose intensity falls off only very slowly over a period of 10 to 15 minutes. Such a glowing fluid in a small test-tube partly exposed to the beam from the carbon arc will have the luminescence completely suppressed in the exposed region in a few

seconds. In fact one may notice a slight inhibitory effect of the arc in 1.5 seconds, which is unmistakable in 2 seconds and marked in 3 seconds. If the tube, partly exposed to the light beam, with consequent suppression of luminescence, is allowed to stand in the dark, the luminescence again reappears somewhat in the suppressed region, but never becomes as bright as the luminescence in the unexposed parts of the tube. The recovery of luminescence is not due to diffusion of more unexposed material into the illuminated area, as a tube of glowing luciferin and luciferase *completely* exposed to illumination until its luminescence is practically extinguished will partially recover in the dark. The effect of light is thus reversible, at least in part.

Not only is it possible to demonstrate the inhibitory effect of light on luminescent material in solution, quite apart from cells, but one may show very simply that light affects the luciferin and not the luciferase. Two tubes of luciferin are prepared, one completely exposed to the carbon arc for several seconds, and the other kept in darkness. When luciferase is now added simultaneously to these tubes in the dark, the exposed tube gives only a faint light while the unexposed one gives a bright luminescence. The converse experiment, exposure of a tube of luciferase to the carbon arc while a second tube of luciferase is kept in the dark, results in the emission of an equally bright luminescence from each tube on the addition of luciferin in the dark.

If the beam of light is allowed to pass through a Nicol prism before striking the test-tube, its inhibitory effect is reduced. That is not surprising since the Nicol transmits only 38 to 40 per cent of the light. I am inclined to attribute the reduction in effectiveness to the absorption and not to the polarization of the light, although I have as yet made no special experiments to determine if one may explain the effect quantitatively by absorption in the Nicol prism.

The far ultra-violet (wave-lengths shorter than 3,000 Å. u.) plays no particular part in the inhibition. A quartz test-tube of luminescent luciferin and luciferase is only very slightly inhibited after $2\frac{1}{2}$ minutes exposure to the light from an iron spark[1] at 3 cm. distance. And the

[1] A disruptive condenser discharge between iron terminals. The apparatus is described by Andrews (Andrews, W. S., *Gen. Elec. Rev.*, 1916, April). It is very convenient for demonstrating ultra-violet fluorescence. The instrument was loaned to me by Mr. Andrews to whom I express my sincere thanks.

inhibition is just as marked when a piece of window glass (opaque to wave lengths less than 3,200 Å. u.) is inserted in the path of the light. Negative effects with far ultra-violet are further seen by studies with the mercury arc. The quartz mercury arc (an old Cooper-Hewett using 3.9 amperes at 72 volts = 280 watts) at a distance of 15 cm. gives as good an inhibition of *Cypridina* luminescence after 15 seconds in a glass test-tube as in a quartz test-tube. Moreover, if we screen out the near ultra-violet by a chlorine-bromine gas filter[2] in a quartz vessel, which lets pass only red, yellow, green, blue (4,358 Å. u.), and far ultra-violet we find only slight inhibition in 90 seconds exposure. The chlorine-bromine filter is especially transparent to the region between 2,550 and 2,650 Å. u. and red, yellow, and green, but opaque to violet (4,078 Å. u. and 4,046 Å. u.) and near ultra-violet (between 4,000 and 3,000 Å. u.). As I shall presently show that red, yellow, and green light has no inhibiting effect, the slight amount of inhibition with the chlorine-bromine filter must be due to the 4,358 Å. u. line.

Since the far ultra-violet is ineffective in inhibiting *Cypridina* luminescence, I have made a study of inhibition in carbon arc light filtered through various glass screens whose transmission is known. The results are tabulated below. For convenience the test-tubes of luminescent luciferin were exposed exactly 15 seconds to an illumination of approximately 8,600 foot candles. Without any filter this illumination appreciably inhibits luminescence in about 2 seconds. The experiments all indicate that light from 4,600 to 3,800 Å. u. is the effective light and that green-blue, green, yellow, red, and infra-red are without action.

For instance the No. 8 filter allows over 75 per cent of all red, yellow, green, and green-blue wave-lengths to pass and 10 per cent of 4,700 Å. u. but only 1.6 per cent of 4,600 Å. u. and none of 4,500 Å. u. The No. 8 filter lets through no light that will inhibit *Cypridina* luminescence under the conditions above mentioned. The No. 3 filter differs from No. 8 only in allowing 40 per cent of 4,600 Å. u., 4 per cent of 4,500 Å. u., and no 4,400 Å. u. to pass. Nevertheless we get inhibi-

[2] Kindly loaned to me by the Hanovia Chemical and Manufacturing Company to whom I express my sincere thanks.

tion of *Cypridina* luminescence behind this filter. Therefore, I place the longer wave-length limit about 4,600 Å. u.

On the other hand, No. 18. the ultra-violet filter, which lets pass a narrow band of ultra-violet with a maximum of about 20 per cent

TABLE I.

Inhibition of Cypridina Luminescence by Light Passing through Wratten Filters.

Filter.	Maximum transmission (figures in μμ).	Inhibition of luminescence.
No. 2 esculin.	Absorbs ultra-violet; transmits all visible.	+
" 3 yellow.	" violet to 450; " rest.	+
" 8 "	" " " 460; " "	−
" 15 "	" " and blue to 510; transmits rest.	−
" 17 "	" " transmits much 350, 10 per cent of 388, and much 470 to red end.	+
" 18 ultra-violet.	Absorbs all but ultra-violet around 350.	−
" 22 orange.	" blue end to 550; transmits rest.	−
" 25 red.	" " " " 590; " "	−
" 29 "	" " " " 610; " "	−
" 36 violet.	Transmits 18 per cent around 410; absorbs rest.	+
" 49 A blue.	" 28 " " " 440; " "	+
" 50 blue.	" 14 " " " 460; " "	Very slight.
" 54 green.	" 0.93 " " " 550; " "	−
" 55 "	" 72 " " " 520; " "	−
" 61 "	" 52 " " " 530; " "	−
" 62 "	" 14 " " " 530; " "	−
" 70 red.	" red to 560; " "	−
" 71 "	" " " 610; " "	−
" 72 orange.	" 4 per cent around 610; " "	−
" 73 yellow-green.	" 8 " " " 570; " "	−
" 74 green.	" 15 " " " 530; " "	−
" 75 blue-green.	" 19 " " " 490; " "	−
" 76 violet.	" 9.5 " " " 440; " "	−*
" 88 infra-red.	" infra-red and 5 per cent of 700; " "	−
G 584 J copper blue.	Strong transmission in blue.	+
" 585 L blue-purple.	85 per cent at 380; 80 per cent at 400.	+
" 586 A purple.	85 " " " 380; 50 " " " 400.	+
" 586 A W "	Like G 586 A but very dense.	−

* Very slight after 1½ minutes exposure.

transmission at 3,500 Å. u. completely prevents inhibition of *Cypridina* luminescence by arc light, while No. 36 which lets pass a narrow band of violet with 18.7 per cent maximum transmission at 4,000 Å. u. allows a little inhibitory light to pass. No. 17 transmits 10

per cent of 3,800 Å. u. and gives some inhibition. The shorter wave-length limit must be somewhere near 3,800 Å. u.

Infra-red radiation which has so marked an effect in inhibiting the phosphorescence of ZnS, CaS and other phosphors, does not inhibit *Cypridina* luminescence, as the experiment with No. 88 filter indicates, and other tests which I have made using iodine in carbon disulfide to absorb the visible.

Cypridina emits light over a spectral range from 4,150 to 6,500 Å. u. and it will be seen that some of these wave-lengths coincide with those we have just found to have an inhibiting effect upon luminescence. We may ask whether *Cypridina* luminescence could possibly inhibit itself. When one considers the difference in illumination between the carbon arc and the *Cypridina* luminescence, a detectable self-inhibition seems hardly likely, but it was thought worth while to test the matter. Accordingly two small test-tubes, *A* and *B* were fixed within two larger test-tubes and the latter filled with a glowing mixture of luciferin and luciferase. In one of the small test-tubes (*A*) water was placed, and in the other (*B*) *Cypridina* luciferin. At the proper time *Cypridina* luciferase was added to *B* so as to produce a bright luminescence which lasted for about 15 seconds and was then completely quenched by adding acid to the *B* tube. Thus the luminescent mixture in the large test-tube surrounding *B* had been exposed to *Cypridina* luminescence while the luminescent mixture surrounding *A* had not. However, both luminescent mixtures were observed to be equally bright, so I conclude that any self-inhibition of luminescence by *Cypridina* light is too slight to be detected.

Finally I have thought it worth while to test the inhibiting effect of light on other chemiluminescences. Using the large carbon arc light providing about 15,000 foot candles, I have observed no quenching effect on the chemiluminescence of phosphorus in air (1 minute exposure), lophin in hot alkaline alcohol (2 minutes exposure), pyrogallol + H_2O_2 oxidized with hemoglobin (10 seconds exposure), or chlorphenylmagnesium bromide[3] in air (2 minutes exposure). The latter compound was placed in a watch-glass and the beam directed on its surface by a silver-under-glass mirror. The illumination was not 15,000 foot candles but possibly 85 per cent of this.

[3] A sample of this compound was kindly presented to me by Dr. R. T. Dufford of the University of Missouri.

CONCLUSIONS.

The luminescence of *Cypridina* luciferin-luciferase solution is inhibited by illumination from a carbon arc of 15,000 foot candles in between 1 and 2 seconds. The blue to violet rays are the effective ones, the limits lying somewhere around 4,600 Å. u. to 3,800 Å. u. The luciferin, not the luciferase, is the substance affected by the light. The effect is partially reversible in the dark. The chemiluminescences obtained by oxidizing phosphorus, lophin, and chlorphenylmagnesium bromide are not inhibited by light under the above conditions.

BIBLIOGRAPHY.

Allman, G. I., 1862, *Proc. Roy. Soc. Edinburgh*, iv, 518.
Harvey, E. N., 1924–25, *J. Gen. Physiol.*, vii, 331.
Heymans, C., and Moore, A. R., 1923–24, *J. Gen. Physiol.*, vi, 273.
Massart, J., 1893, Sur l'irritabilité des noctiluques, *Bull. sc. France et Belgique*, xxv.
Moore, A. R., 1923–24, *J. Gen. Physiol.*, vi, 403.
Parker, G. H., 1920, *J. Exp. Zool.*, xxxi, 475.
Zacharias, O., 1905, *Biol. Centr.*, xxv, 20.

THE EFFECTS OF LIGHT ON LUMINOUS BACTERIA.

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It is a well known fact that sunlight and artificial light inhibit the luminescence of some luminous animals, notably Ctenophores. I have recently discovered (1925) that a glowing mixture of *Cypridina* luciferin and luciferase in a test-tube will have its luminescence suppressed by light from a carbon arc, and it is possible to show that the inhibitory effect is exerted upon the luciferin and not upon the luciferase. I am inclined to regard this phenomenon as possibly of more general occurrence than heretofore believed and of fundamental significance in connection with bioluminescence. One is reminded of the action of red and infra-red radiation in suppressing the phosphorescence of ZnS and other phosphors.

The question arises whether luminous bacteria show any suppression of luminescence in light, that might be compared with inhibition in Ctenophores or in *Cypridina*. Suchsland (1898) found no effect of sunlight on luminous *Bacterium phosphorescens*, kept under glass and water to prevent warming. McKenney (1902) kept cultures of *Bacillus phosphorescens* in darkness, alternate daylight and darkness, and exposed continuously to a 16 c.p. electric lamp at 2 feet during a period of 48 hours. He found no differences in intensity of luminescence in the three cultures.

On the other hand Lode (1908) reports that luminous *Vibrio rumpel* is very sensitive to sunlight and Beijerinck (1915) finds *Photobacter splendidum* killed by direct sunlight. It is well known (Beijerinck (1915) and Gerretsen (1915)) that ultra-violet radiation from a quartz mercury lamp will kill luminous bacteria, although the luminescence does not immediately disappear as a result of the exposure. The ultra-violet light produces changes in the organism which ultimately lead to its death, together with failure of luminescence.

The above mentioned experiments have not been carried out in the proper manner to demonstrate an inhibiting effect of light which may disappear quickly. The sort of suppression we are interested in is an immediate inhibition of luminescence after radiation, with possible recovery in the dark, *i.e.* a direct effect of light upon the luminous reaction proceeding within the luminous bacteria, not an effect of light on the growth of the organism or a lethal effect finally resulting in death. Accordingly, the following experiments have been devised to demonstrate any immediate inhibitory effect of light upon luminescence in bacteria.

The light from a carbon arc (soft cored 13 mm. diameter carbons, at right angles, using 15 amperes at 55 volts = 825 watts) in a dark house, after passing through 60 mm. water, is condensed to a slightly converging beam by a lens 135 mm. in diameter. The beam passes through a black tube with a screen at the end containing a slit 8 mm. wide × 20 mm. long, so that all light is excluded from the dark room except a narrow band, 8 mm. × 20 mm. in whose path a small test-tube of luminescent bacterial emulsion may be placed. The illumination in the region of the test-tube is about 15,000 foot candles, much greater than sunlight at noon in summer (10,000 foot candles), but the light had passed through glass so that all deleterious ultra-violet rays were removed. A camera shutter for rapid screening of the beam was placed before the test-tube so that it could be examined very quickly after exposure. Since the beam is narrow (8 mm.) only a narrow area of the test-tube need be exposed to light, the portions above and below the beam remaining in comparative darkness. Thus we have the opportunity of examining contiguous areas of bacteria one of which has been illuminated and the other not, a condition which should bring out any changes in luminescence intensity which may appear. The bacterium used was *Bacterium phosphorescens*[1] isolated from fish by Mr. T. F. Morrison, to whom I am deeply indebted for culturing the organism. The emulsion of the organisms in sea water was made so dilute that it was not necessary to bubble air through the test-tube containing them during the course of an experi-

[1] The experiments were repeated with another form isolated by me and gave identical results.

ment. There was enough oxygen dissolved in the sea water to maintain the luminescence.

Emulsions of luminous bacteria exposed to 15,000 foot candles, as described above, for periods, of time varying from 15 seconds to 4 minutes, show no trace of inhibition when examined perhaps $\frac{1}{5}$ of a second after the illumination was cut off. They also show no change in light intensity when exposed in a quartz test-tube to a narrow pencil of light from a quartz mercury arc (Cooper-Hewett 72 volts, 3.9 amperes) at a distance of 15 cm. As *Cypridina* luminescence is inhibited under the same conditions in a few seconds, we must conclude that luminous bacteria show no suppression of luminescence as a result of illumination.

Thinking that light might bring about a suppression of luminescence of very short duration in bacteria, I have examined the organisms in

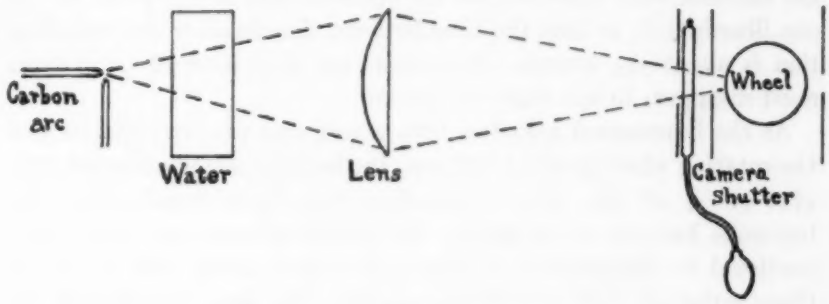

FIG. 1.

a phosphoroscope of the revolving disc type, first described by Becquerel and used in somewhat modified form by many others since (see Andrews, 1920). Fig. 1 shows the general arrangement of the apparatus. Light from a small carbon arc in a dark house is made parallel by a quartz lens, screened and diaphragmed, and allowed to strike the edge of a flat-faced wheel as a circle of light, 6 mm. in diameter. The soft cored, 8 mm. diameter carbons used about 200 watts and gave about 5,700 foot candles on the wheel. If white paper is wound on the wheel and the wheel, attached to the shaft of a motor, is rapidly rotated, one can see very clearly the phosphorescence of the paper by examining the rotating disc through a window from the side opposite to that which the light beam strikes. The paper and many

other substances luminesce for a considerable time after illumination, so that when we examine the luminous bacteria on the wheel it is necessary to place them on some non-phosphorescent material. Black felt cloth was finally selected as a proper medium, wound on the wheel and painted with a dense emulsion of luminous bacteria to form a band about 18 mm. wide. When the wheel is rapidly rotated many of the bacteria are thrown off by centrifugal force but enough of them remain to present a luminous band when viewed through the window. As the beam of light is 6 mm. in diameter and strikes the middle of the luminous band, 18 mm. wide, any changes in luminescence intensity of the bacteria should be plainly apparent in the center of the luminous field.

I have failed to detect any effect of illumination on the luminescence of the bacteria. The wheel revolved about 100 times per second and the bacteria were examined on the opposite side of the wheel to the one illuminated, so that the time between illumination and examination is about $\frac{1}{200}$ second. Therefore, any inhibiting effect of light must disappear in less than $\frac{1}{200}$ second.

As the illuminated area is 6 mm. across and the circumference of the rotating wheel is about 144 mm. the bacteria are illuminated only $\frac{6}{144}$, or $\frac{1}{24}$ of the time. Assuming that light would affect the luminous bacteria according to the Bunsen-Roscoe law (effect proportional to illumination $\times$ time), they were really exposed to an illumination of $\frac{5,500}{24}$ or 229 foot candles. In later experiments an illumination of 15,000 foot candles was used, so that the effective illumination during rotation was $\frac{15,000}{24} = 625$ foot candles. No effect could be observed in the bacteria after 1.5 minutes exposure. Again assuming the Bunsen-Roscoe law to hold, our exposure in this experiment is $625 \times 90 = 56,250$ foot candle seconds, amply sufficient to suppress the inhibition of *Cypridina* luminescence.

CONCLUSION.

A conservative statement would therefore be that luminous bacteria show no changes in luminescence as a result of illumination by 625 foot candles for 1.5 minutes when examined $\frac{1}{200}$ of a second after exposure, and none as the result of illumination by 15,000 foot candles for 6 minutes when examined $\frac{1}{5}$ of a second after exposure.

BIBLIOGRAPHY.

Andrews, W. S., 1920, *Gen. Elec. Rev.*, xxiii, 856.
Beijerinck, M. W., 1915, *Folia microbiol.*, iv, 15.
Gerretsen, F. C., 1915, *Centr. Bakt., 2. Abt.*, xliv, 660.
Gerretsen, F. C., 1920–21, *Centr. Bakt., 2. Abt.*, lii, 353.
Harvey, E. N., 1924–25, *J. Gen. Physiol.*, vii, 679.
Lode, A., 1908, *Centr. Bakt., 2. Abt.*, xxii, 421.
McKenney, R. B., 1902, *Proc. Biol. Soc. Washington*, xv, 213.
Suchsland, E., 1898, Festschrift zur 200-jährigen Jubelfeier der Franckeschen Stiftungen, Halle, 87.

THE RELATIVE IMPORTANCE OF pH AND CARBON DIOXIDE TENSION IN DETERMINING THE CESSATION OF CILIARY MOVEMENT IN ACIDIFIED SEA WATER.

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The activities of all living cells are so profoundly influenced by the hydrogen ion concentration of their surroundings that for a number of years physiologists have concerned themselves very extensively with determinations of the pH of physiological fluids without sufficient recognition of the fact that two solutions of exactly the same pH may have totally different effects upon the internal reaction of cells exposed to them. It has been shown by Jacobs (1–3), in the case of systems containing CO_2 and bicarbonate or free ammonia and ammonium salts, that external and internal reactions frequently do not run parallel. Intracellular acidity may be produced by alkaline solutions, or intracellular alkalinity by acid ones. The experiments of Smith and Clowes (4, 5) also show that while the reaction of the surrounding sea water is a factor of much importance in determining the rate of cleavage of *Asterias* and *Arbacia* eggs, the effects produced at the same pH are very different according to whether the buffer system used contains much or little free CO_2. Very recently, similar results have been obtained with rabbit intestine by Fraser (6) and with human leucocytes by Pearse (7).

In the present series of experiments an attempt has been made to determine whether the same principles apply to cilia, which because of their small volume and relatively enormous surface might be expected to show less contrast between external and internal effects than is the case with most other protoplasmic structures. The general result of these experiments is to indicate that, while the pH

of the surrounding medium is a factor of much importance in determining cessation of ciliary movement, the actual character of the buffer system concerned—in particular its CO_2 tension—is a factor of equal or even greater importance.

The material used was obtained from the gills of the mussel, *Mytilus edulis*. The behavior of the cilia of the gill filaments was followed in

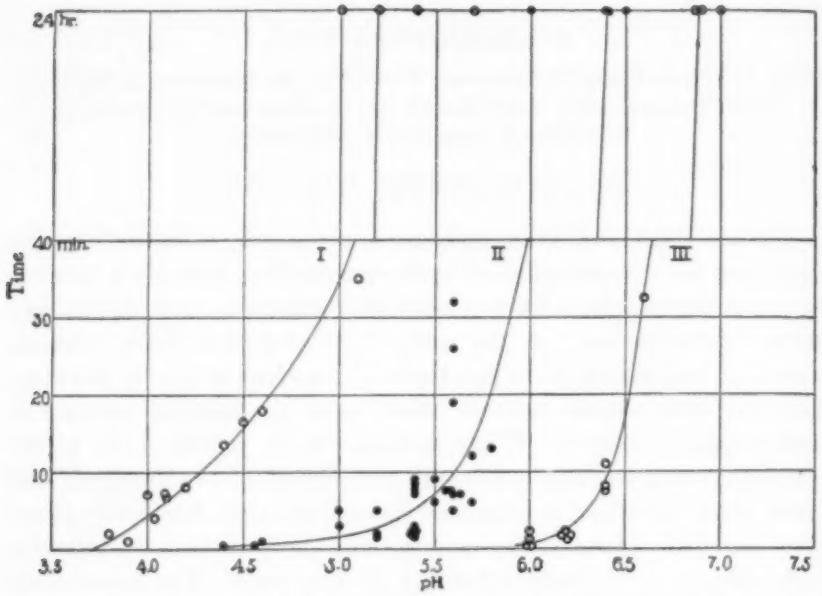

FIG. 1. Curves showing the effect of various CO_2 tensions upon the length of time required for cessation of ciliary beat in *Mytilus* gill. The abscissa represents the pH; the ordinate represents time in minutes up to 40 minutes, above which, at the top of the figure, are indicated 24 hour values.

I. Low CO_2 tension. (Sea water acidified and aerated.)

II. Moderate CO_2 tension. (Sea water acidified, but not aerated.)

III. High CO_2 tension. (Sea water and $NaHCO_3$, acidified, but not aerated.)

sea water of low, medium, and high CO_2 tension and of varying pH values. The method employed in preparing the solutions was essentially that of Smith and Clowes (5). The experiments were carried out in small, glass-stoppered bottles of 35 to 40 cc. capacity which were filled and kept closed to prevent the escape of CO_2. The desired ranges of pH and CO_2 tensions were obtained by using (I) sea water

which, after the addition of sufficient N/2 HCl, was aerated in an automatic shaker for 1 hour to reduce the CO_2 tension to that of the atmosphere; (II) sea water to which HCl was added without subsequent aeration; and (III) a 2.5 per cent solution of M/2 $NaHCO_3$ in sea water to which HCl was also added without subsequent aeration. Solutions II and III were prepared in the bottles used for the experiments in order that the loss of CO_2 might be minimized. Since the indicators employed (brom-phenol blue, methyl red, brom-cresol purple, and brom-thymol blue) were shown by control experiments to be non-toxic in the slight concentrations required for adjusting the reactions of the solutions, they were added directly to the latter.

The procedure employed in the experiments was the following. Three or four small pieces of *Mytilus* gill were introduced into a bottle nearly filled with the solution to be used and containing only sufficient indicator to make the necessary pH adjustment; HCl was then quickly added, the bottle stoppered, and the contents mixed by gentle shaking. The pH was determined by comparison with a set of standard tubes. The pH values here given must, for strict accuracy, be corrected for the salt error due to the use of sea water, but for comparative purposes they are sufficiently exact. After various lengths of time the bottles were opened and the pieces of gill were removed for immediate microscopic examination. Because it often appeared that the cilia in different portions of the gill were affected to different extents by the acid medium, the time determined was that required for the cessation of the beat of the smaller terminal cilia, which were very easily seen in profile. When a bottle to which HCl had been added was opened, its contents were discarded after the first examination, since the CO_2 content of the solution could then no longer be controlled.

In the accompanying graph the various experiments made have been grouped together to show not merely the average values obtained but the range of variation encountered. In the case of single experiments made on material from the same gill the data when plotted gave fairly smooth curves similar in form to those which have, for the sake of clearness, been drawn to represent the general trend of the observations. It will be seen from inspection of Curve I that the pH as such is evidently a factor of importance, since in this case the CO_2

tension was not allowed to vary but was at all times that of the room air. That the pH of the solution is not the only, or indeed the most important factor, however, is shown by a comparison of this curve with the other two. It will be observed that in the first curve (where the CO_2 tension is low) that at a pH of 5.6, for example, the beat continued for over 24 hours; it ceased in the second (where the CO_2 tension is medium) at the same pH in about 30 minutes or less; and in the third (where the CO_2 tension is high) it ceased instantly. With each of the given CO_2 tensions a zone exists which may be considered as a critical region for the cessation of the ciliary beat. The upper limit of this zone is represented by the lowest pH at which the beat continues for 24 hours; the lower limit is the pH which stops the cilia at once. The values for this region for each CO_2 tension would then be approximately as follows:

Low CO_2 tension	pH 3.7–5.2
Moderate CO_2 tension	pH 4.4–6.4
High CO_2 tension	pH 5.9–6.9

Evidently, therefore, in the case of *Mytilus* cilia, as in the experiments of Smith and Clowes on echinoderm eggs, the CO_2 tension has an effect of its own.

Whether carbon dioxide produces its characteristic effects on cilia by increasing the intracellular acidity as has been supposed by Jacobs (2) to be the case in some of his experiments, or acts in some other more specific manner, must for the present be left undetermined. It is at any rate clear that in work done on this and similar material where the pH of the sea water is changed by the addition of acid the results will be of little value if care is not taken to control at the same time the carbon dioxide tension of the medium.

SUMMARY.

The length of time that cilia from the gills of *Mytilus* continue to beat in acidified sea water depends to some extent on the pH of the solution but to a greater extent on its carbon dioxide tension.

I wish to express my thanks to Dr. M. H. Jacobs for advice most kindly given throughout my study of this problem.

BIBLIOGRAPHY.

1. Jacobs, M. H., *Am. J. Physiol.*, 1920, li, 321.
2. Jacobs, M. H., *Am. J. Physiol.*, 1920, liii, 457.
3. Jacobs, M. H., *J. Gen. Physiol.*, 1922–23, v, 181.
4. Clowes, G. H. A., and Smith, H. W., *Am. J. Physiol.*, 1923, lxiv, 144.
5. Smith, H. W., and Clowes, G. H. A., *Am. J. Physiol.*, 1924, lxviii, 183.
6. Fraser, L. McP., *Am. J. Physiol.*, 1925, lxxii, 119.
7. Pearse, H. E., *Arch. Int. Med.*, 1925, xxxv, 347.

CRITICAL INCREMENT FOR OPERCULAR BREATHING RHYTHM OF THE GOLDFISH.

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I.

The respiratory movements of fishes are about doubled in frequency when the temperature increases from 10° to 20°C. (*Cf.* Kanitz, 1915, 1923; Przibram, 1923.) The data for various species (due to Bethe, Baglioni, Babák, Kuiper, and others) show, however, considerable irregularity in the Q_{10} ratios for corresponding temperature intervals. This might be due to the occurrence of distinct controlling reactions in different parts of the temperature range, undetected unless numerous observations are made; or to the accidental effect of intrinsic variability in the frequency of movements; or to the existence of real differences in the critical increment for respiratory movements in different species or in the same species under different conditions.

To secure a basis for interpretation of the mechanism controlling respiratory rhythm more detailed observations are required. We have measured the frequency of opercular rhythm in the goldfish. This animal was chosen because measurements are available permitting estimation of the temperature characteristic for its gaseous exchanges.

The method of observation was similar to that already employed with the frog (Crozier and Stier, 1924–25). Each individual was confined in a flat-sided glass vessel immersed in a thermostat. The fish-container, partially filled with water, was closed by a sealed cover carrying a thermometer and tubes permitting air to be bubbled through the water. Air supplied in this way was first brought to thermostat temperature. The opercular breathing rhythm was watched

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through a small opening in a yellow paper envelope surrounding the thermostat. By this procedure certain troublesome variations in breathing frequency, often associated with swimming movements, are greatly reduced. It was desired to obtain the critical increment in the absence of experimental interference with the animal and therefore we did not remove the eyes.

For some time before the commencement of the observations the fishes had been living in balanced aquaria at room temperature (not far from 16°). These animals averaged 6 cm. in length, and withstood somewhat lower oxygen concentrations than found for larger goldfish by Gardner and King (1922). Comparative tests in which the

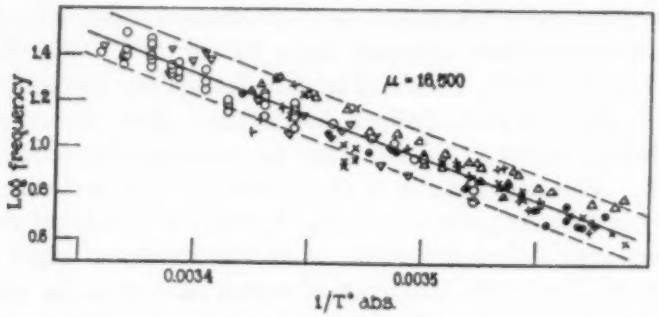

FIG. 1. The frequency (100 divided by time for 10 movements) of rhythmic opercular movements in six goldfish (different symbols) at different temperatures, when the pectoral fins are not obviously moving. The four most divergent points are single observations, the majority of the others, averages of four or more closely concordant readings. The critical increment is 16,500 calories. (The observations pertaining to one individual (black circles) are divided by 1.34.)

oxygen concentration was varied between 0.68 and 6.2 cc. per liter showed no significant effect of oxygen concentration upon breathing frequency, except that with high oxygenation the opercular movements may be quite in abeyance for considerable periods.

II.

In the absence of visual stimulation periods of swimming activity tend to alternate with periods of quiescence. Three types of breathing frequency must be separated if a significant temperature characteristic is to be deduced. During active swimming the opercular

rhythm is irregular, and immediately after a period of vigorous movement the resting rhythm is accelerated. When the animal is quiet there is a corresponding greater frequency if the pectoral fins are in gentle movement. Reliable figures must therefore be obtained from

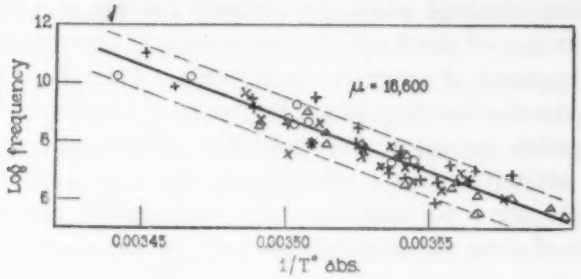

FIG. 2. Frequency of rhythmic opercular movements in four individuals starved 11 to 13 days. The readings with one animal (white circles) have been multiplied by 1.18. Other series of readings not plotted are fully consistent with these. The increment is the same as with non-starved animals (Fig. 1), and the latitude of variation at constant temperature is the same, but the mean frequency at any temperature is higher.

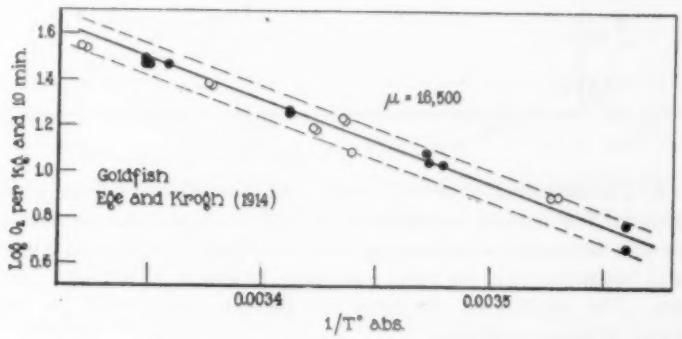

FIG. 3. The critical increment for velocity of oxygen consumption by goldfish is 16,500 calories. Data from Ege and Krogh (1914); white circles, narcotized with urethane.

quiet animals, with the pectorals at rest. It is not easy to secure large numbers of such readings above 15°C., although relatively easy to do so below that temperature.

Data from six experiments with different individuals are given in Fig. 1. These manifest the kinds of regularities already noted for

other phenomena controlled by temperature. The linear relationship between *log frequency of movements* and *reciprocal of absolute temperature* yields $\mu = 16{,}500$ calories.

This magnitude of the critical increment also fits satisfactorily the observations obtained when the pectoral fins are moving (Fig. 4). The occurrence of rhythmic fin movements is accompanied by increased frequency of opercular movements. Starvation for several weeks produces alterations in the frequency of breathing movements, and somewhat increases the variability at constant temperature, but also without seriously affecting the thermal constant (Fig. 2). These facts serve to show that the temperature characteristic is indicative of some fundamental property of the respiratory center.

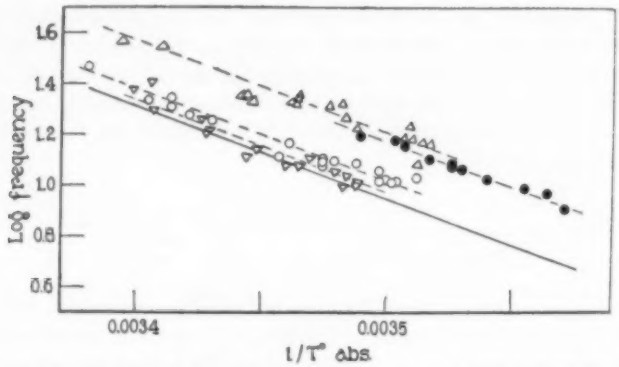

FIG. 4. The frequency of opercular movements in goldfish poised in mid water with pectoral fins moving rhythmically is higher at each temperature than in animals with motionless pectorals (Fig. 1); but the critical increment is unchanged. The solid line is drawn in the same position as in Fig. 1. The plotted points are averages. The amount of the frequency increase varies with the individual considered (different symbols).

III.

The most significant series of measurements from which the temperature characteristic for metabolic oxidations in the goldfish may be estimated is that by Ege and Krogh (1914). The critical increment (Fig. 3) is 16,500 cal.[1] There is thus a quantitative corre-

[1]In a previous paper (Crozier, 1924–25, p. 200) this value was erroneously given as 16,100. The analyses by Gardner, King, and Powers (1922) provide

spondence between oxygen utilization and frequency of breathing movements. It is unlikely that the frequency of opercular movements determines the rate of total oxygen consumption. On the other hand, the critical increment for opercular rhythm serves provisionally to classify the controlling reaction among the respiratory oxidations which so frequently exhibit this increment (Crozier, 1924–25). From this standpoint the reaction respectively controlling opercular rhythm in the goldfish (μ = 16,500) and pharyngeal rhythm in the frog (μ = 8,800; Crozier and Stier, 1924–25, *b*) may perhaps be supposed to represent two distinct but possibly catenary reactions in the metabolism of cells composing the respiratory center.[2] The situation in these vertebrates would then be similar to that demonstrated among insects (Crozier and Stier, 1924–25, *a*). It might be predicted that, as with insects, under suitable experimental treatments the breathing rhythm of the goldfish and frog could be caused to exhibit new critical increments, such as characterize otherwise concealed reactions in the metabolism of the controlling nervous elements in the two forms. This prediction is being tested.

IV.

The nature of the variation in frequency of opercular movements, at constant temperature, requires brief consideration. The pertinent facts are these: the latitude of variation tends to be a well defined constant fraction of the mean frequency, at each temperature; the incidence of pectoral movements accelerates the opercular rhythm, but there is clear indication that the "central discharge" controlling the movements is thereby merely pitched at a new level,—for the critical increment is sensibly the same; a similar result is obtained under moderate starvation. It is possible to consider that these variations are similar in origin to the shift in frequency without change

μ = 20,000, approximately, for velocity of O_2 utilization and of CO_2 output; it is not clear that the effects of movements are adequately excluded and the data show considerable irregularity, but the older measurements of Jolyet and Regnard (1887) are not wholly inconsistent with them.

[2] From a quite different standpoint this conclusion regarding the control of respiratory rhythm has been stated by Gesell (1923, 1924–25); *cf.* also McClendon (1917).

of increment which we have observed in the quiescent frog. A full theory of these effects awaits further testing, but it is believed that they permit the assumption of definite views regarding the mechanism of variability in certain kinds of central nervous processes. If it be supposed that the reactions controlling discharge from the respiratory center are specifically synaptic in locus, the effective mass of a governing catalyst might be varied according to the influx of nerve impulses from other parts of the central nervous system. Changes of this kind would abruptly alter the frequency of the observed activity, without changing its critical increment.

One method of testing this conception consists in determining the frequency and temperature characteristic for respiratory movements in amphibians after removal of the inhibitory control exercised by the forebrain. The results will be given in a following paper.

SUMMARY.

The temperature characteristic for opercular breathing rhythm in the quiescent goldfish is 16,500 calories. The reactions governing the frequency of breathing movements in vertebrates appear to be constituent links in the oxidative metabolism of the controlling cells.

CITATIONS.

Crozier, W. J., 1924–25, *J. Gen. Physiol.*, vii, 189.

Crozier, W. J., and Stier, T. B., 1924–25, *a*, *J. Gen. Physiol.*, vii, 429; 1924-1925, *b*, *J. Gen. Physiol.*, vii, 571.

Ege, R., and Krogh, A., 1914, *Internat. Rev. Hydrobiol. u. Hydrograph.*, vii, 48.

Gardner, J. A., and King, G., 1922, *Biochem. J.*, xvi, 729.

Gardner, J. A., King, G., and Powers, E. B., 1922, *Biochem. J.*, xvi, 523.

Gesell, R., 1923, *Am. J. Physiol.*, lxvi, 5.

Gesell, R., and Hertzman, A. B., 1924–25, *Proc. Soc. Exp. Biol. and Med.*, xxii, 298.

Jolyet, F., and Regnard, P., 1887, *Arch. physiol. norm. et path.*, iv, series 2, 584.

Kanitz, A., 1915, Temperatur und Lebensvorgänge, Berlin.

Kanitz, A., 1923, in Oppenheimer, C., Handbuch der Biochemie des Menchen und der Tiere, Jena, 2nd edition, ii, 200.

Kestner, O., and Plaut, R., 1924, in Winterstein, H., Handbuch der vergleichende Physiologie, Jena, ii, pt. 2, 901.

McClendon, J. F., 1917, Physical chemistry of vital phenomena, Princeton and London.

Przibram, H., 1923, Temperatur und Temperatoren im Tierreiche, Leipsic and Vienna.

TEMPERATURE CHARACTERISTIC FOR HEART BEAT FREQUENCY IN LIMAX.

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In order to obtain information as to the types of critical thermal increments for heart beat frequency in a variety of animals, and thus, it is to be expected, some knowledge of the kinds of controlling reactions, it was found desirable to reinvestigate certain more or less typical forms. The present note contains data upon the relation between temperature and frequency of cardiac pulsations in the slug *Limax maximus*.

The animals were obtained from a greenhouse, since at this season (March) they are inactive out-of-doors. Some days before the observations were made the anterior portion of the mantle was cut away. Each individual was studied by placing it about midway in a glass tube of just sufficient diameter to accommodate the slug. This tube (2 feet long) was surrounded by a much larger tube through which water at the desired temperature was passed. The temperature of the animal's surroundings could then be adequately controlled when the outer tube was then wrapped with cotton, except in the immediate vicinity of the slug. A sensitive, calibrated thermometer was adjusted in the smaller tube, with its bulb very close to the head of the slug. Light from a stereopticon lamp, passing first through heat filters and a diaphragm, was sufficiently intense to make visible the pulsations of auricle and ventricle. This illumination had itself no systematic effect on the heart beat. The removal of the anterior portion of the pigmented mantle increased visibility. For each observation the time for ten beats was measured. Precautions were taken to reduce errors from faulty thermal adaptation. It was not found necessary to arrange for a constantly renewed supply of air within the tube.

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Results with several individuals are given in Fig. 1. The logarithm of the frequency of heart beats is inversely proportional to the absolute temperature. The type of variation and the consistency in the comparative records of different specimens are similar to those encountered in cases previously studied (Crozier, 1924–25; Crozier and Federighi, 1924–25, *a* and *c;* Crozier and Stier, 1924–25). The critical increment (temperature characteristic) derived from Fig. 1 is $\mu = 16{,}300 \pm 200$ cal.

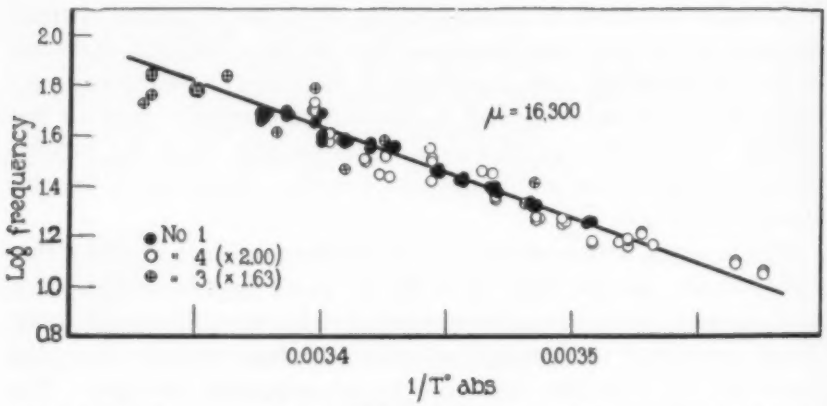

FIG. 1. The frequency of the heart beat in *Limax maximus* as function of temperature; three individuals (different symbols). The points are in some instances single observations, but in many cases represent two or more coincident readings. The linear relationship satisfies the equation $ln \frac{K_2}{K_1} = \frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$, where K_2 and K_1 are the frequencies at the respective absolute temperatures.

This value of the temperature characteristic of the neurogenic heart beat is of interest because it agrees closely with that derived from the consideration of certain somatic movements of *Limax* (Crozier and Federighi, 1924–25, *b*). The observations given in Fig. 1 refer to the heart beats of animals in a state of quiescence. If, however, the slug be moving—turning slowly, or creeping,—the frequency of the beats is immediately accelerated to an extent depending largely upon the vigor of the movements. So far as can be judged, the critical increment remains the same, but it is very difficult to secure sufficient observations under strictly comparable degrees of muscular

activity. A certain amount of the variation evident in Fig. 1 may be traceable to slight movements of the tentacles and mouth; one individual (No. 3), the most variable, was more restless than the others.

The temperature characteristic obtained with *Limax* may be compared with that calculated from published observations upon the heart beat in certain other molluscs.

Some interesting although not very numerous observations by Lang (1910) upon the heart beat of *Helix* at different seasons yield

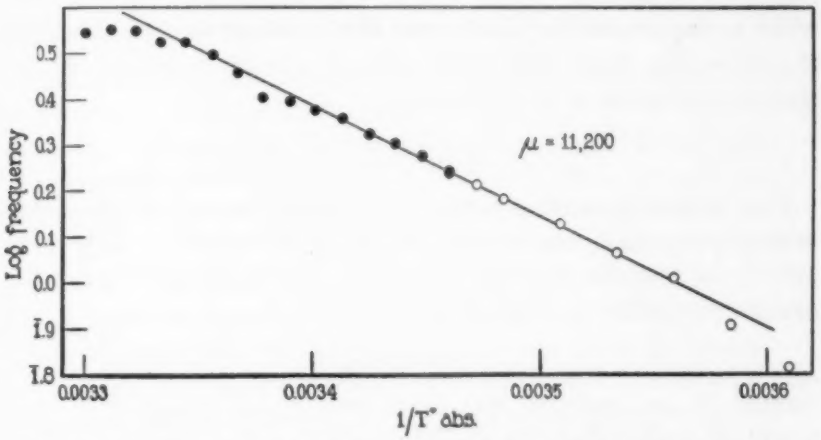

FIG. 2. Frequency of the heart beat in *Anodonta;* data from Koch (1916–17). Between 8° and 26° a satisfactory fit is obtained with $\mu = 11{,}200$. Two series of observations are adjusted for comparison by dividing the frequencies in one set (white circles) by 2.38.

increments 7,900 (June), 11,300 (January), and 16,000 (February, March). This type of finding is suggestive because temperature characteristics of these particular magnitudes are associated with respiratory phenomena in a great variety of instances (Crozier, 1924–25; Crozier and Stier, 1924–25). It may be suggested that the mechanism determining heart frequency is different at different seasons, in *Helix*. The fact that incomplete experiments of our own with *Limax* in December gave $\mu = 7{,}900 \pm$ is confirmatory. Snyder (1906–07) gives data upon the heart beat of *Phyllirrhoe*, at four temperatures; from these $\mu =$ about 10,700. Bachrach and Cardot

(1923) measured the heart beat frequency in embryos of *Arion;* the mean frequencies at three intermediate temperatures yield μ = 12,700. A few observations by Crozier and Arey (1919) on the heart of *Chromodoris* give μ = 12,300. In these and in some other instances it is uncertain to what extent the magnitude of the deduced increment may be influenced by movements of the animals, or by the existence of undetected changes in μ on either side of some critical temperature. Koch's (1916–17) data for heart beat in *Anodonta* (Fig. 2) yield over the major portion of the temperature range the increment 11,200 cal. These facts tend to suggest that the reactions which in one or another case control the frequency of the heart beat of molluscs are those involved in a general system of processes constantly represented in living matter.

SUMMARY.

The temperature characteristic for the frequency of the heart beat in quiescent *Limax maximus* is μ = 16,300 calories. This value agrees well with that obtained for certain neurogenic activities of the parietal musculature of this gastropod.

CITATIONS.

Bachrach, E., and Cardot, H., 1923, *Compt. rend. Soc. biol.*, lxxxix, 788.
Crozier, W. J., 1924–25, *J. Gen. Physiol.*, vii, 189.
Crozier, W. J., and Arey, L. B., 1919, *J. Exp. Zool.*, xxix, 261.
Crozier, W. J., and Federighi, H., 1924–25, *a*, *J. Gen. Physiol.*, vii, 137; 1924–25, *b*, *J. Gen. Physiol.*, vii, 151; 1924–25, *c*, *J. Gen. Physiol.*, vii, 565.
Crozier, W. J., and Stier, T. B., 1924–25, *J. Gen. Physiol.*, vii, 429.
Koch, W., 1916–17, *Arch. ges. Physiol.*, clxvi, 281.
Lang, A., 1910, Festschrift zum 60. Geburtstag R. Hertwigs, iii.
Snyder, C. D., 1906–07, *Am. J. Physiol.*, xvii, 350.

THE KINETICS OF STARVATION.

II. The Loss of Weight in Pigeons Subsisting on Water Alone.

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(Accepted for publication, April 22, 1925.)

In a previous paper[1] it was shown that the single cycle symmetrical form of the autocatalytic law could not by itself be made to represent the changes of weight during the entire course of starvation in pigeons suffering from partial inanition. By the addition of two autocatalytic curves, however, a procedure which recognized the cyclical nature of the process, it was found that very good agreement between theoretical and observed losses in weight could be obtained. Consequently, it was suggested that the course of starvation is governed by the rate of destruction of body protein and that it is modified by the amount and by the rate of destruction of reserve tissues. Plausible as this conclusion is, it could not be applied to the phenomena of complete inanition without experimental verification. The present paper, accordingly, deals with the application of the earlier principles to the problem of pure starvation.

THEORY.

At the outset it may be stated that the following analysis is only incidentally concerned with fitting a curve to the loss of weight observed in partial and in complete inanition. Strictly curve-fitting enterprises have been previously attempted.[2-4] The primary object

[1] Wetzel, N. C., *J. Gen. Physiol.*, 1924–25, vii, 269.

[2] Morgulis, S., Fasting and undernutrition, New York, 1923.

[3] Benedict, F. G., A study of prolonged fasting, *Carnegie Institution of Washington, Pub. No. 203*, 1915, 73–79.

[4] Jackson, C. M., The effects of inanition and malnutrition upon growth and structure, Philadelphia, 1925.

has been to present a function which will not only have the power of meeting the observational results, but which, when completely analyzed, will also convey an impression as to the nature of the fundamental reactions participating in the process and will finally afford some quantitative measure of these reactions. These postulates are satisfied by a suitable application of the law of autocatakinesis.[5]

Only a short time ago, in further studies of the population problem, Pearl[6] derived a general equation for the mathematical expression of this law free from the restrictions of the single cycle form. This equation may be written:

$$y = \frac{k}{1 + me^{F(t)}} \tag{1}$$

in which y represents the increments on the ordinate, k the asymptote of the curve, and $F(t)$, some undefined function of time expanded by means of Taylor's theorem into an infinite series so that

$$me^{F(t)} = e^{a_1 t + a_2 t^2 + a_3 t^3 + \ldots + a_n t^n}$$

the factor m being equal to e^{a_0}. In this form the expression provides for any number of cycles which may be either symmetrical or asymmetrical in nature. The labor of fitting this curve to cases where the changes in adjacent cycles are nearly symmetrical is reduced by using a simpler form substantially like that employed in the previous communication:[1]

$$y = d + \frac{k}{1 + me^{ka't}} \tag{2}$$

in which d is the upper asymptote of the earlier cycle and a' gives the rate of change in the epoch under consideration. Henceforth, the discussion is limited to the general relation represented by equation (1).

If then, the whole course of partial starvation can be denoted by the addition of two symmetrical autocatalytic curves, it should like-

[5] Lotka, A. J., Elements of physical biology, Baltimore, 1925, 76. (This author recalls the suggestion of Wo. Ostwald as to the preference for the above term which is free from the chemical implication of *autocatalysis*.)

[6] Pearl, R., Studies in human biology, Baltimore, 1924.

wise conform to the general modification. As a matter of fact, the necessary constants for equation (1) were calculated from the data on partial inanition previously reported, as well as from the more recent data on several groups of birds which received nothing but water. The resultant curves gave a most remarkable fit to the observed values of weight loss, considering the fact that over thirty observations are represented by a five constant equation. Only curves of total starvation are shown in Fig. 1 since the results of partial inanition were for all purposes the same as before.

Although equation (1) is a purely mathematical relation which defines a curve so closely approximating the curve of weight loss observed in fasting pigeons during complete as well as during partial inanition, it is desirable to have such a relation denote something more definite from the standpoint of the physiology and the physical chemistry of the process. It must be clear that as long as an equation contains a term such as Taylor's series this object will not be easily attained. As it stands the above expression has far more to commend it than the several types of merely empirical relations, such as the conic sections or the inverse logarithmic equations[7] that have been proposed to meet the requirements of the starvation curves in different animals. Equation (1) does express the kinetic relationships of a definite type of chemical reactions and it makes provisions for the cyclical character of certain processes the extent of which is limited by the value of k. The difficulty in employing this expression, however, lies in the fact that the coefficients of time in the exponential expansion do not easily lend themselves to interpretation in terms of physicochemical conceptions. There is, moreover, the difficulty of clearly differentiating between the successive cycles or phases as regards the rate of decomposition during the course of the process, a matter of considerable importance in the problem under discussion.

For these reasons it has seemed more appropriate to express the exponential function directly in terms of the constants of the participating reactions. It has been shown that the entire process can be

[7] The chief objection to these functions is that the weight diminishes indefinitely as t increases. The equation suggested by Morgulis[2] (p. 89) when rearranged becomes the familiar monomolecular law of chemical change.

represented completely by an equation which may be written in the form

$$y = \frac{A}{1 + e^{-k_1(t - t_1)}} + \frac{B}{1 + e^{-k_2(t - t_2)}} \tag{3}$$

in which the constants have the same significance as previously described. Letting

$$Z = A + B$$

$$P = 1 + e^{-k_1(t - t_1)}$$

$$Q = 1 + e^{-k_2(t - t_2)}$$

the exponential factor in the denominator of (1) may be easily transformed by making the proper substitutions leading to the result

$$me^{F(t)} = \frac{Q\left[\frac{Z}{B}(P - 1) + 1\right] - P}{Q\left(\frac{Z}{B} - 1\right) + P} = \theta. \tag{4}$$

The equation giving the loss of weight during the entire course of starvation then becomes

$$y = \frac{Z}{1 + \theta} \tag{5}$$

in which y represents tissue loss in gm. or in percentage units, Z the maximum loss attainable, and θ the function of time determined from (4). Similarly, it is evident that the actual weight curve is given by

$$M_t = \frac{M_0[\theta + (1 - \alpha)]}{1 + \theta} \tag{6}$$

in which M_0 and M_t are the weights at the beginning of the starvation period and at time t respectively, and α is the fraction of tissue destroyed, namely, $\frac{Z}{M_0}$

Such a transformation, evidently, overcomes the difficulties to which reference has been made. It must be noted, however, that this

procedure is valid only when the successive cycles are symmetrical or nearly so. Should marked asymmetry develop in any cycle the

TABLE I.

Showing the Observed Weight Loss Compared to the Loss Calculated by Means of Equation (5) and the Corresponding Values of the Exponential Function in Equations (1) and (4).

Time.	$me^{\Sigma a_n t^n}$	θ	Weight loss.	
			Calculated.	Observed.
days			*gm.*	*gm.*
0	14.880	11.300	—	0
1	9.900	7.900	24.9	20
2	6.680	5.580	33.8	34
3	4.400	4.060	43.9	45
4	3.300	3.020	55.2	57
5	2.700	2.420	65.0	67
6	2.100	1.900	76.5	76
7	1.700	1.570	86.0	84
8	1.430	1.320	95.0	91
9	1.200	1.200	101.0	100
10	1.050	1.030	109.0	107
11	0.914	0.940	114.0	114
12	0.795	0.830	121.1	121
13	0.705	0.730	128.2	129
14	0.619	0.650	134.5	137
15	0.539	0.564	142.0	144
16	0.464	0.466	151.5	153
17	0.399	0.385	160.2	159
18	0.333	0.316	168.8	167
19	0.280	0.255	176.5	173
20	0.223	0.199	185.1	181
21	0.183	0.170	189.8	188
22	0.135	0.109	200.0	197
23	0.100	0.099	202.1	202
24	0.067	0.076	206.1	206
25	0.033	0.057	210.8	213
26	0.026	0.042	213.0	215
27	0.016	0.032	215.8	218
28	0.010	0.024	216.9	219
29	0.007	0.018	219.0	221
30	0.003	0.002	221.0	222

general form (1) alone would meet the requirements. Any cyclical and nearly symmetrical function of the foregoing type can then be

analyzed either by means of the identical relations (3) and (2) or by means of the θ transformation and (5). The latter method has the additional advantage of combining the summations necessary for the former procedures into a single expression similar in form to the general law.

Owing to the nature of the present problem certain further restrictions must be imposed on both equations (4) and (5). In the first place, all negative values of time are immediately eliminated since the onset of starvation must necessarily coincide with the point $t = 0$ on the x axis. Again, it can be seen from inspection of the elements in equation (4) that as t increases, P as well as Q must diminish, remain positive, and approach $+1$ as a limit. The ratio $\frac{Z}{B}$ is likewise restricted to positive values greater than 1, otherwise the amount of tissue decomposed would be greater than the amount initially available. Under these conditions θ remains positive and approaches 0 while y approaches the upper asymptote Z. For diminishing values of t, on the other hand, the curves of P, Q, and θ ascend hyperbolically, intercepting the y axis well above 0. The solution for y at this point, accordingly, gives a result that cannot be interpreted as tissue loss. It could, perhaps, be taken to indicate the potential instability of the system near this parameter immediately prior to a displacement of equilibrium.[8] Nevertheless, any increment in y however small must require a correspondingly small but definite interval of time for the change. Thus, the limitation of t to positive values greater than 0 enables equation (5) to describe the changes in mass with a precision that can hardly be due to chance alone.

RESULTS.

1. Experimental.—The loss of weight observed in one of the groups of twenty pigeons which received nothing but water is recorded in the last column of Table I and is charted by small circles along Curve 1 in Fig. 1, the curve itself passing through the points calculated by means of equation (5), the values of which are set up in the fourth column of the table. Curve 2 is plotted from another group of twenty

[8] Lotka,[5] Chapter XXII.

birds which were also on water alone, merely to demonstrate the identity of the processes and to present another instance of the singular agreement between observed and theoretical results. To date, the curves of about 100 birds thus treated have been studied and have been found to be similar in all essential respects to those of Fig. 1. For the present, the group represented in the table and by Curve 1 is selected for discussion. The remainder, including those of Curve 2, have been treated somewhat differently in order to study the effect of various feedings preliminary to starvation. These data have not yet been completely analyzed.

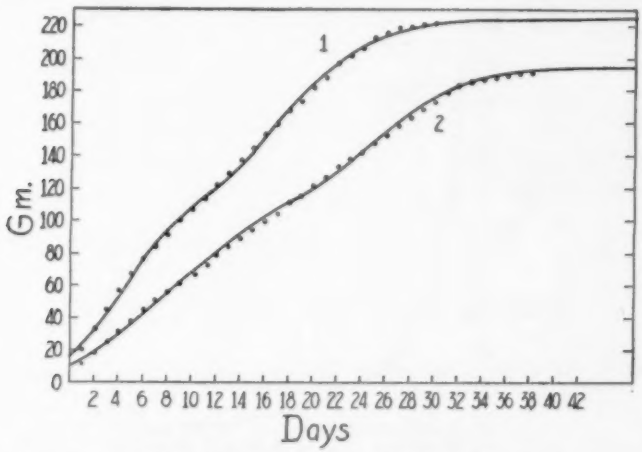

FIG. 1. Two curves showing the loss of weight in pigeons on water alone. The curves themselves pass through the points calculated by means of equation (5). The small dots represent the experimentally observed loss of weight. Curve 1 corresponds to the data in the table.

The mean weight of the selected group on the initial day of the experiment was 429 ± 2.88 gm. Particular care was taken to choose individual pigeons which were as nearly alike in respect to weight as possible, so that the active masses undergoing change might likewise be more comparable. Consequently, the standard deviation of the group was only 19.1 ± 2.02 gm. In order to give still greater reliability to the final results the number of birds placed in a single group was relatively large, in this particular instance twenty, and in no case below fifteen. The probable error of the mean, therefore, during the

entire experiment fluctuated closely about a value of 1 per cent of the mean. The weights were recorded to the nearest gm. at 24 hour intervals. The average weight of the group was taken as the weighted mean of those pigeons still alive on any particular day.

The maximum relative loss observed on the 40th and final day of the experiment was 53.4 per cent. The mean duration of life was found to be 25.2 ± .908 days. The former value, however, appears to be somewhat higher than a critical analysis of the data would war-

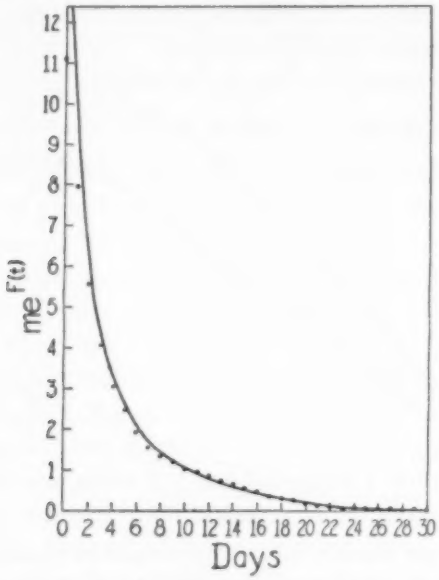

FIG. 2. The variation of the exponential factor with time. The plotted points are the values of θ in Table I, the curve representing the values of $me^{F(t)}$.

rant, due to the fact that two birds survived as long as the 34th and 40th days respectively. Since the curve rapidly becomes asymptotic to its limiting value in the final stages, the geometric mean between the average loss on the 25th and 40th days was taken as a first approximation to the most probable figure for the maximum relative weight loss, namely 51.5 ± .613 per cent, precisely the loss observed on the 30th day of starvation which itself is close to the geometric mean between the average and final lethal day.

2. Computation of Results.—If the curve of the function $me^{F(t)}$,

in which $F(t)$ is expanded by Taylor's theorem, be regarded as the more exact representation of this function it is of interest to note in Fig. 2 how closely the values of θ approximate the curve. The greatest deviations occur in the region where the slope is the steepest and have, accordingly, little effect on the corresponding values of y. The constants for this curve were computed by Pearl's method for the coordinate points:

$$t_1 = 1 \qquad y_1 = 20$$
$$t_2 = 9 \qquad y_2 = 100$$
$$t_3 = 17 \qquad y_3 = 159$$
$$t_4 = 25 \qquad y_4 = 213$$
$$t_5 = 33 \qquad y_5 = 222$$

and were found to be:

$$\beta_1 = -2.1138 \qquad a_0 = 2.3126$$
$$\beta_2 = 3.2384 \qquad a_1 = -0.41366$$
$$\beta_3 = -5.4767 \qquad a_2 = 0.024157$$
$$a_3 = -0.00068393$$

The whole equation then became:

$$y = \frac{222}{1 + e^{2.3126 - .41366t + .024157t^2 - .00068393t^3}}$$

The constants for equation (3) were calculated by Robertson's method.[9] They are:

$$k_1 = 5.58 \qquad t_1 = .128 \text{ mos.} \qquad A = 100 \qquad Z = 222$$
$$k_2 = 3.80 \qquad t_2 = .575 \text{ "} \qquad B = 122 \qquad \frac{Z}{B} = 1.86$$

Values of θ for each day were then determined directly from equation (4). Finally, the theoretical weight loss, y, was calculated by means of equation (5). Experience has shown that the adjustment of the two cycles can be made almost as closely by inspection of the curves plotted from the experimental data as by the method of least squares, a process involving an undue amount of time and labor.

Thus, the evidence submitted in the present study would tend to show that the fundamental kinetic relations governing the processes

[9] Robertson, T. B., The chemical basis of growth and senescence, Monographs on experimental biology, Philadelphia and London, 1923.

of complete and partial starvation are essentially alike. This evidence, in both its experimental and mathematical aspects is wholly in accord with the results of the earliest investigations into this problem made nearly a century ago by Chossat,[10] who observed that the changes in mass are by no means uniform throughout the period of tissue destruction.

SUMMARY AND CONCLUSIONS.

1. The loss of weight in pigeons subjected to prolonged starvation on water alone may be accurately represented by an appropriate form of the law of autocatakinesis.

2. This function, within proper limitations, gives a definite indication as to the nature and character of the fundamental reactions involved. In addition, it provides quantitative measures of the relative progress and importance of these reactions.

3. Analytically, the process of starvation consists of two nearly symmetrical cycles, corresponding chemically to the destruction of the carbohydrate-fat reserves and to the decomposition of body protein.

4. A suitable transformation overcoming certain difficulties inherent in the general form of the law is discussed and applied to the data reported.

[10] Chossat, C., Recherches expérimentales sur l'inanition, *Mém. Acad. roy. sc., Paris*, 1843, 13–18.

DIURNAL CHANGES IN THE ACIDITY OF BRYOPHYLLUM CALYCINUM.*

By FELIX G. GUSTAFSON.

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(Accepted for publication, April 25, 1925.)

In studies on the hydrogen ion concentration gradient and total acidity in plants it was noticed that these varied greatly in *Bryophyllum calycinum* with the light conditions of the day; on a cloudy day the H ion concentration and total acidity were very high, while on a bright sunny day they were low.[1] From this it was inferred that there might be diurnal changes in these concentrations. Reference to literature shows that in 1813 Benjamin Heyne[2] reported that the tropical plant *Bryophyllum* (*Cotyledon*) *calycinum* tasted as sour as sorrel early in the morning, but by noon was tasteless. This was verified by Link, with litmus paper. He also found that light was responsible for the disappearance of the acid. (This is quoted by Kraus.[3]) Mayer,[4] Kraus, Warburg,[5] and others have shown that this plant is acid during the night and that the acidity decreases during

* Paper from the Department of Botany of the University of Michigan, No. 229.

[1] Gustafson, F. G., Total acidity compared with actual acidity of plant juices, *Am. J. Bot.*, 1924, xi, 365.

[2] Heyne, B., On the deoxidation of the leaves of *Cotyledon calycina*, *Tr. Lin. Soc., London*, 1815, xi, 213.

[3] Kraus, G., Ueber die Wasservertheilung in der Pflanze. IV. Die Acidität des Zellsaftes, *Abhandl. naturforsch. Ges. Halle*, 1883, xvi, 143.

[4] Mayer, A., Ueber die Bedeutung der organischen Säuren in den Pflanzen, *Landw. Versuchs-Stationen*, 1875, xviii, 428.

[5] Warburg, O., Ueber die Bedeutung der organischen Säuren für den Lebensprozess der Pflanzen (speciell der sog. Fettplanzen), *Untersuch. bot. Inst. Tübingen*, 1886–88, ii, 53.

the day time. Spoehr[6] and Richards[7] have found the same to be true for *Opuntia versicolor*. For an extended review of the literature on acidity in plants reference should be made to Richards' paper.

In all of this work there are no references to changes in the H ion concentration or actual acidity of the plants studied. And as far as the writer is aware there has been no study of the H ion concentration in plants extending over a period of 24 consecutive hours. There are occasional statements to the effect that the H ion concentration is slightly different at 10:00 a.m. from what it is at 5:00 p.m., but these differences are usually so small as to be without significance.

In previous experiments[8] it had been found that there is a H ion concentration gradient in *Bryophyllum* and that the greatest difference in acidity on a cloudy day and a bright day is in the young parts of the plant. From this it was apparent that young plants of the same age and size should be compared. For each set of experiments plants were propagated from leaves, all from the same parent plant, and they were all treated in exactly the same manner. From these, plants were selected that were of the same size and with the same number of leaves. Whole tops were used and ground up in the usual way. Either clear juice or that containing chloroplasts and small pieces of cellular tissue could be used. In preliminary experiments it was established that centrifuging or settling out had no effect on either the actual or total acidity. The juice could be used at once or allowed to stand several days before using, without any change either in actual or total acidity, as long as fungi had not started to grow in the liquid. This was a very interesting point, showing a difference between *Bryophyllum* and other plant juices. In most plant juices there is a decided change in H ion concentration within an hour or even half an hour after it has been expressed. It may also be mentioned that boiling for a few minutes had no effect upon the acidity.

After a sufficient number of plants had been obtained several experi-

[6] Spoehr, H. A., Photochemische Vorgänge bei der diurnalen Entsäuerung der Succulenten, *Biochem. Z.*, 1913, lvii, 95.

[7] Richards, H. M., Acidity and gas interchange in cacti, *Carnegie Institution of Washington, Pub. No. 209*, 1915.

[8] Gustafson, F. G., Hydrogen ion concentration gradient in plants, *Am. J. Bot.*, 1924, xi, 1.

ments were conducted in which the actual and total acidity were determined at various times during a period of 24 hours or over. The H ion concentration was determined by potentiometer and the total acidity by electrometric titration as described in previous papers.[1,8] The graph shown in Fig. 1 is typical of these first experiments conducted when the day was sunny. At 10 o'clock in the morning, when the experiment was started, the actual as well as the total acidity was very high as most plants go, but not excessively so for *Bryophyllum calycinum*. The total acidity decreased steadily and was at its lowest at a few minutes before 4 in the afternoon; after that time it slowly increased till late the next morning. This experiment was conducted the 10th of December with the sun setting at about 5 o'clock, and at 4 it no longer reached the plants in the greenhouse. During the 6 hours of daylight from 10 to 4 the H ion concentration had decreased from pH 4.15 to pH 5.37, and the hydroxide required to neutralize 10 cc. of the plant juice from 11 cc. 0.1365 N NaOH to 3.1 cc. 0.1365 N. The plants were kept in the dark greenhouse during the night and measurements taken at 8 and 12 p.m. and 6:30 a.m. During this time the temperature remained nearly constant. The maximum actual and total acidity was reached at 10 the next morning. The pH was then 3.90, slightly lower than the preceding morning, while the hydroxide requirement was higher; 12.1 cc. 0.1365 N NaOH being required to neutralize 10 cc. of the plant juice. At 12 o'clock, when the experiment was discontinued, the pH of the juice was 4.43, somewhat lower than that of the day before at the same time. The difference in the actual and total acid concentration at corresponding times during the 2 days is undoubtedly due to unequal brightness of the preceding day. It has been reported by other investigators, and the writer has noticed repeatedly, that on a morning following a very bright day the concentration of acid is much higher than on a morning following a cloudy day. This difference is probably due to the difference in amount of photosynthesis, although the writer has no data to substantiate this surmise. From the experiment cited it is evident that in *Bryophyllum calycinum* there is a fluctuation in H ion concentration within a wide range, corresponding to total acidity changes as previously reported.

Similar experiments were conducted when the day was dark and cloudy. Fig. 2 is representative of such an experiment, started at 4 o'clock in the morning. At that time the pH was 3.82 and 8.0 cc. of 0.1365N NaOH were required to neutralize 10.0 cc. of the plant juice. Determinations were made at various times during the day and early part of the night. As usual the H ion concentration decreased as the day advanced, reaching a minimum at about 5 in the afternoon. There was, however, a much smaller decrease than during a sunny day; the lowest H ion concentration being only pH 4.40 as compared with pH 5.37 on a sunny day. The total acid con-

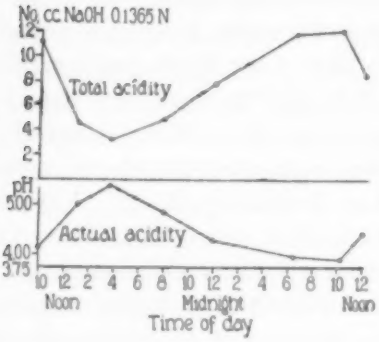

FIG. 1.

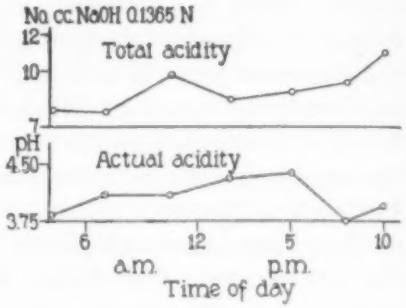

FIG. 2.

FIG. 1. Diurnal changes in total and actual acidity in *Bryophyllum calycinum* on a sunny day. The curves represent a single typical experiment.

FIG. 2. Diurnal changes in total and actual acidity in *Bryophyllum calycinum*, on a dark, cloudy day. The curves represent a single typical experiment.

centration did not decrease below that found at the beginning of the experiment, and in fact it actually increased slightly during the day.

From the experiments represented by Fig. 2 it seems as if the decrease in H ion concentration is produced by a condition which does not reduce the total amount of acid. Bright sunlight reduces both, but a smaller amount of light does not diminish the total acidity, while it slightly decreases the actual acidity; and when the light intensity is still further diminished there is no reduction in either kind of acidity. An increase occurs in absence of light. In a previous paper[1] the writer has pointed out that a plant which has a high H ion con-

centration may have a lower total acidity than another one with a somewhat lower H ion concentration. The experiment during a cloudy day may lead one to believe that at different times different acids may be responsible for the acid conditions of a plant. It may be that on a cloudy day acids are produced, which are only slightly ionized, and on a bright day acids with a higher degree of ionization may be formed.

Mayer[9] established that one of the acids formed in the *Crassulaceæ* is isomalic. Besides oxalic acid Spoehr[6] also found malic acid in *Opuntia versicolor*. From Figs. 1 and 2 it seems that the amount of buffer action is not always the same which might indicate that different acids are present. Fig. 1 records an experiment in which the H ion concentration at 10:00 a.m. was pH 4.15 and 11.0 cc. of 0.1365 N NaOH were required to neutralize 10 cc. of the plant juice. With another plant, for which the results are shown in Fig. 2, the H ion concentration was greater at 4:00 a.m., namely pH 3.82, yet only 8.0 cc. of the same hydroxide were required to neutralize 10 cc. of this plant juice. In the latter plant the total acid was less than in the former, although its H ion concentration was considerably greater.

Figs. 3 and 4 show the titration curves of several organic acids, of combinations of them, and also of juice from *Bryophyllum*.

It is evident at once that when the total acid concentration in any of these acids or their combinations approaches that of the *Bryophyllum* juice the H ion concentration is greater than that of the juice. It seems to the writer that the formation of acid salts, yielding a lower concentration of H ions, may account for the greater part of the total acidity found in the juice.

There has been considerable discussion and speculation as to the formation and subsequent destruction of the acids. Most writers are of the opinion that the acids are due to incomplete oxidation of the carbohydrates, brought about perhaps by lack of oxygen in the succulent parts of the plant. The problem of the destruction of the acid is as yet unsettled, though it is agreed that the acids are not directly used in photosynthesis. Light, temperature, and oxygen are all supposed to influence the deacidification process.

[9] Mayer, A., Ueber die Sauerstoffausscheidung einiger Crassulaceen, *Landw. Versuchs-Stationen*, 1878, xxi, 277.

Experiments were conducted to determine the effect of light on the destruction of the acid substances. Fig. 5 represents one of these experiments. Similar plants were chosen and divided into two groups, one of which was placed outside the greenhouse where the sunlight was as bright as in the house, but the temperature was considerably lower, while the other group was left in the greenhouse.

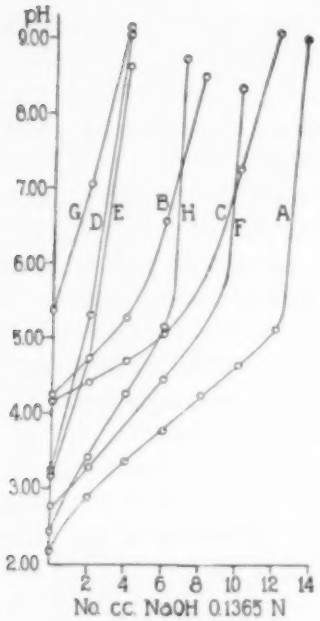

FIG. 3.

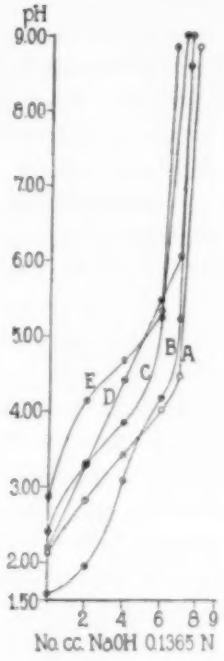

FIG. 4.

FIG. 3. Titration curves: *A*, 0.2 N malic acid; *B*, 0.1 N malic acid; *C*, a mixture of 1/3 0.4 N malic acid, 1/3 0.1 N glycine, and 1/3 0.1 M asparagine; *D*, 1/3 0.1 N malic acid, 1/3 0.1 N glycine, and 1/3 0.1 M asparagine; *E*, 1/4 0.1 N malic acid, 1/4 0.1 N acetic acid, 1/4 N glycine, and 1/4 0.1 M asparagine; *F*, *Bryophyllum* juice collected 9:50 a.m.; *G*, *Bryophyllum* juice collected 3:45 p.m.; and *H*, *Bryophyllum* juice collected at midnight. All plants were in bright sunlight during the day, and all the plants formed part of the experiment represented in Fig. 1. Each curve represents a single titration.

FIG. 4. Titration curves of some organic acids: *A*, 0.1 N tartaric; *B*, 0.1 N oxalic; *C*, 0.1 N formic; *D*, 0.1 N citric; and *E*, 0.1 N acetic. Each curve represents a single titration.

At 11:00 p.m. the plants from the outside were placed in a dark room at a temperature of about 18°C., where they remained overnight. The top curves of Fig. 5 give the temperature at the time of determination of acidity. It will be seen from this that the temperature in the greenhouse remained fairly constant, fluctuating between 25° and 31°C., but outside there was a greater variation, from 18–4.5°, as well as a much lower temperature.

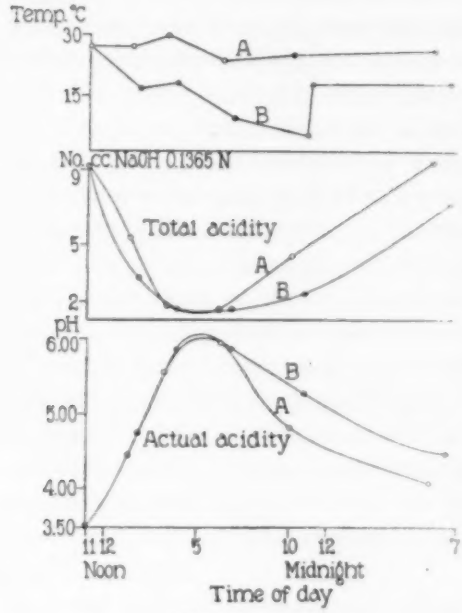

FIG. 5.

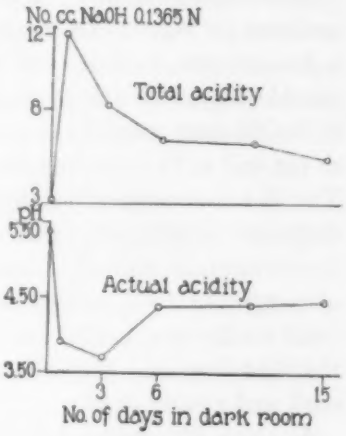

FIG. 6.

FIG. 5. In this figure is shown the influence of light and temperature on deacidification in *B. calycinum*. *A* represents results from plants in the greenhouse and *B*, from plants out of doors. The upper set of curves gives the temperature in the greenhouse and outside, during the time of the experiment; at 11:00 p.m. the plants were transferred from the outside at a temperature of 4.5° to a dark room at a temperature of about 18°. The middle set of curves gives the total acidity at various times during the period of the experiment, while the lower set gives the H ion concentration at corresponding times. The curves represent a single typical experiment.

FIG. 6. Total and actual acidity conditions in *B. calycinum* in continuous darkness for 15 days at a temperature of 18–20°C. The curves represent a single typical experiment.

Both the actual and total acidity decreased at approximately the same rate in the plants kept in the warm greenhouse and the plants kept at the lower temperature outside, when the day was bright. All experiments agree in this respect. On a cloudy day with less light the plants in the lower temperature showed a lower rate of deacidification than those in the warm greenhouse.

From Fig. 5 it is evident that light is a much more important factor in the decomposition of acids than is temperature. When the light is intense the temperature does not seem to play any part. One would expect that in the warm greenhouse the deacidification would proceed more rapidly than outside where the temperature is lower. Studies on the temperature effect are to be continued.

Deacidification also takes place in the dark, though very slowly, as shown by Fig. 6. During a period of 15 days, in a dark room having a temperature between 18° and 20°C., the total acidity decreased considerably. At the beginning of the experiment 12 cc. of 0.1365 N NaOH were required to neutralize 10 cc. of the plant juice, while at the end of 15 days only 5.4 cc. of the same hydroxide were needed. The H ion concentration increased to a maximum during the first 3 days and slightly decreased during the next 3 days, but remained approximately constant during the remainder of the experiment, although the total acidity continued to decrease. In the dark the total acidity and actual acidity curves show no correlation, while in the light they do. The absence of correlation is already noticeable on dark and cloudy days.

In these discussions only the decomposition of the acids has been mentioned. It is, however, to be borne in mind that at all times acids are also being formed, and the acid condition at any moment is the resultant of the two opposite processes, and when it has been stated that light accelerates the deacidification, it is merely meant that in bright light more acid is broken down than is being formed; *i.e.*, the point of equilibrium has been changed.

By some investigators it has been stated that an abundance of oxygen aids deacidification. The writer kept plants in a dark room under bell jars to which oxygen was supplied constantly from a tank. In one instance the plants were kept under the above condition for 48 hours, and actual and total acidity determined on these plants and

on controls. The acidity condition of the experimental plants was the same as that of the control. In another experiment the plants were in an incubator at 36°, for 6 hours, oxygen being supplied during this time. In this experiment also the rate of decrease in acid was the same as in control plants at that temperature. Other plants were covered with bell jars into which a stream of hydrogen was constantly passing, reducing the oxygen tension. This in no way affected the results. From these experiments it seems that the oxygen tension outside the plant is not an important factor in the acid decomposition in *Bryophyllum calycinum*.

In his work on *Opuntia versicolor* Spoehr[6] states that the juice is decomposed in light even after it has been boiled for 3 minutes. Similar experiments were conducted with the juice from *Bryophyllum* plants in 100 cc. Erlenmeyer flasks. These flasks were exposed to normal daylight and at night to three 100 watt lamps fitted with reflectors. Juice was obtained from a large plant and the H ion concentration and actual acidity determined at once on a sample. The remainder of the juice was treated as follows:

1. Juice in cotton-stoppered flask, in light for 4 days.
2. Juice in flask with oxygen bubbling through the liquid, in light for 4 days.
3. Juice in flask which was aerated with hydrogen several times a day, in light for 4 days.
4. Juice in flask aerated once or twice a day with carbon dioxide, in light for 4 days.
5. Juice boiled for 3 minutes in flask stoppered with cotton, in light for 4 days.
6. Juice in cotton-stoppered flask in dark incubator at the same temperature as those in light (about 30°) for 6 days.

This experiment was repeated several times and in all trials the H ion concentration and the total acidity were approximately the same under the different treatments and these were practically the same as the fresh juice obtained from the plant. The juice when extracted is not appreciably deacidified under any conditions tried. This is contrary to Spoehr's findings in *Opuntia versicolor*, and it appears that the acid decomposition in *B. calycinum* is connected with the life activities of the plant.

The formation of acid is a normal phenomenon in *B. calycinum* and is undoubtedly due to incomplete respiration, brought about by lack of oxygen and perhaps the slow escape of CO_2. The stomata are few in number, only about 31 to the sq. mm. on the lower side, as compared to 190 in *Pelargonium sp.*, 330 in *Syringa vulgaris*, 625 in *Olea europaea*, etc. The stomata are very small and many are incompletely formed. The leaves are very thick (about .85 mm.), with compact cell arrangement, leaving little space for air cavities. A considerable quantity of CO_2 is formed in the dark.

In the light photosynthesis is rapid, and it is possible that active oxygen may be released in the cells where the acid is stored. The acids may be rapidly oxidized to carbon dioxide and water (or some compound such as formaldehyde) which may be used at once in photosynthesis. Thus light may indirectly aid the decomposition of the acids by supplying the active oxygen, as well as by removing carbon dioxide.

The acid condition of a plant at any moment may be considered as largely determined by light, temperature, and perhaps oxygen, as well as the food in the cells. Of these light is the most important, always tending to decrease the acidity. Upon the food substances (carbohydrates mainly) depends the ultimate amount of acid which may be formed. After 24 hours in the dark the acidity does not appreciably increase, but begins gradually to decrease, as the food supply is used up in respiration. In the dark the acid substances are gradually broken down and are probably used as reserve foods in the respiration of the plant.

SUMMARY.

1. It has been shown that there is a diurnal change in the H ion concentration in *Bryophyllum calycinum* corresponding approximately to the total acidity changes. The H ion concentration increases at night and decreases during the day.

2. Light is the main factor in causing the decrease in acidity, though it also occurs in the dark, but much more slowly.

3. External oxygen tension does not seem to influence the acidity of the plant, nor does it influence the decomposition of the extracted juice.

AN IMPROVED TYPE OF MICROSCOPIC ELECTROCATAPHORESIS CELL.

By JOHN H. NORTHROP and M. KUNITZ.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Accepted for publication, April 28, 1925.)

The cataphoresis cells previously described[1,2] are troublesome to make and have the further disadvantage that they cannot be cleaned with cleaning solution. Dr. D. P. Morgan called the writers' attention to the fact that a flat glass capillary cell could be made by drawing out tubing sidewise as shown in Fig. 1. A 1.5 cm. test-tube is a convenient size to use. The capillary should be about 1.0 by 5 mm. inside dimensions and as uniformly rectangular in cross-section as possible. It is then sealed to short pieces of glass tubing as shown in Fig. 2, and the whole connected with the $Zn\text{-}ZnSO_4$ electrodes as previously described.[2] Slight curvature of the glass may be overcome by sealing cover-slips with Canada balsam to the top and bottom of the cell.

In using the cell the potential drop per cm. in the cell itself must be known. This may be calculated from the cell dimensions as previously described[1] or measured directly. For direct measurement two small platinum electrodes are sealed into the cell about 2 cm. apart, and the potential drop per cm. which occurs in the cell itself is determined by measuring the potential between these electrodes with an electrometer.[3] This measurement may also be made with a potentiometer provided the apparatus is filled with mercury. The cell is assembled and filled with dry mercury. A dry cell is connected in series with the two Zn electrodes and the potential between these electrodes determined with a potentiometer. The potential between the electrodes sealed into the cell is also determined. This gives the propor-

[1] Northrop, J. H., *J. Gen. Physiol.*, 1921–22, iv, 629.

[2] Kunitz, M., *J. Gen. Physiol.*, 1923–24, vi, 413.

[3] A potentiometer cannot be used with an electrolyte solution in the cell, owing to polarization.

tion of the total potential drop which occurs in the cell, *i.e.* if V_T is the potential between the Zn electrodes, V_C the potential between the electrodes in the cell, then, since the electrodes are 2 cm. apart, the ratio of the potential drop per cm. in the cell to the total potential is $\frac{V_C}{2V_T}$. This factor is constant for any one cell and the drop per cm. at any time is therefore $\frac{V_C}{2V_T} \cdot V$, where V is the total voltage applied

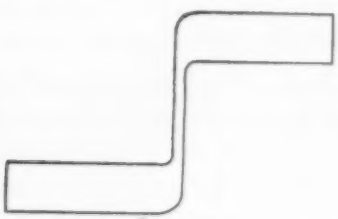

FIG. 1.

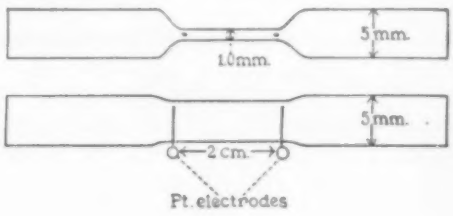

FIG. 2.

FIG. 1. Method of drawing out flat capillary.
FIG. 2. Diagram of cell.

to the Zn electrodes during the measurement. This potential between the two Zn-$ZnSO_4$ electrodes at the ends of the apparatus may be determined with a voltmeter. The arrangements of the zinc electrodes and the other connections are the same as those already described, as is the method of correcting the motion of the particles for the motion of the water in the cell. For small particles an adjustable dark-field condensor may conveniently be used.[4]

[4] The entire apparatus may be obtained from Eimer and Amend, New York.

CRITICAL THERMAL INCREMENTS FOR OXYGEN CONSUMPTION OF AN INSECT, DROSOPHILA MELANOGASTER.

By PAUL RUDBERT ORR.

(*From the Zoological Laboratory, University of Pennsylvania, Philadelphia.*)

(Accepted for publication, May 1, 1925.)

Crozier and others (1, *a* and *b*, 2, *a*, *b*, *c*, 3, 4) in recent investigations on the effect of temperature on biological processes have shown that for certain vital activities the underlying or controlling processes may be treated as systems of "irreversible" first order reactions and that to such processes the equation of Arrhenius (5), $K_2 = K_1 e^{\frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$ should be applied.

For respiratory processes, oxygen consumption and carbon dioxide production, critical thermal increments have been shown by this method of analysis to be of two and possibly three types: μ = 11,500 and 16,100 or 16,700 (Crozier 1, *b*).

The present paper contains results obtained by a similar analysis of data from experiments on the effect of temperature upon the oxygen consumption of pupæ of the "wild type" fruit fly, *Drosophila melanogaster*.

I wish to express my gratitude to Dr. J. H. Bodine whose continuous aid and inspiring criticism has made this work possible.

The pupæ furnishing the data set forth in this paper belong to a basic wild stock of *Drosophila melanogaster*. This stock has been carried along in mass cultures in this laboratory for the last three years.

Oxygen determinations were made by the manometer method previously described (6). Constant temperatures were maintained by means of a modified type of the Freas constant temperature water bath. For temperatures lower than room temperature large coils of copper tubing were placed in the water bath and through

them water at low temperature was slowly circulated. Constant low temperatures were thus maintained to within ±.1°C.

Equal numbers of pupæ (usually 50), previously sterilized by alcohol and then washed in distilled water, were used in each determination. In some experiments individual lots of pupæ were subjected to the entire series of temperatures (0.5°C. to 35°C. inclusive) while in other experiments separate lots of comparable pupæ were used at only one temperature. No marked differences were noted in the general nature of the results from these differently tested pupæ. Since during early pupal development the chief processes taking place within the animal (histolysis) are quite different from those of the last 2 days (differentiation, growth, etc.) it is desirable to use pupæ all of the same age. Separate experiments were, therefore, carried out with pupæ of 1, 2, 3, and 4 days of age and with an otherwise identical history as regards temperature and cultural conditions. As shown in Fig. 1 no marked differences in critical thermal increments for oxygen utilization of the pupæ of different ages were found, thus indicating that no significant differences seem to exist between critical thermal increments for oxygen utilization during these different periods.

The figure shows the relation between temperature and oxygen utilization as found for all pupæ of all ages. The values of the critical thermal increments (11,500 above 15°C., and 16,800 below 15°C.) are strikingly similar to those pointed out by Crozier (1, *b*) for respiratory processes in various plants and animals. These critical thermal increments show that rates of oxygen utilization can be described accurately in terms of the Arrhenius equation. It is to be noted in the figure that a "break" in the value of μ occurs at approximately 15°C. This point corresponds almost exactly with that similarly pointed out for numerous organisms by Crozier and others (1, *a* and *b*).

The values for critical thermal increments for oxygen utilization in *Drosophila*, as found in these experiments, are of quite different magnitude than those obtained by Crozier (1, *b*) from data of Loeb and Northrop for velocity of development and of death for the same organism. This fact seems to lend further support to the conception of a lack of fundamental relation between the critical thermal increment for oxygen utilization and for measurements of growth, etc.

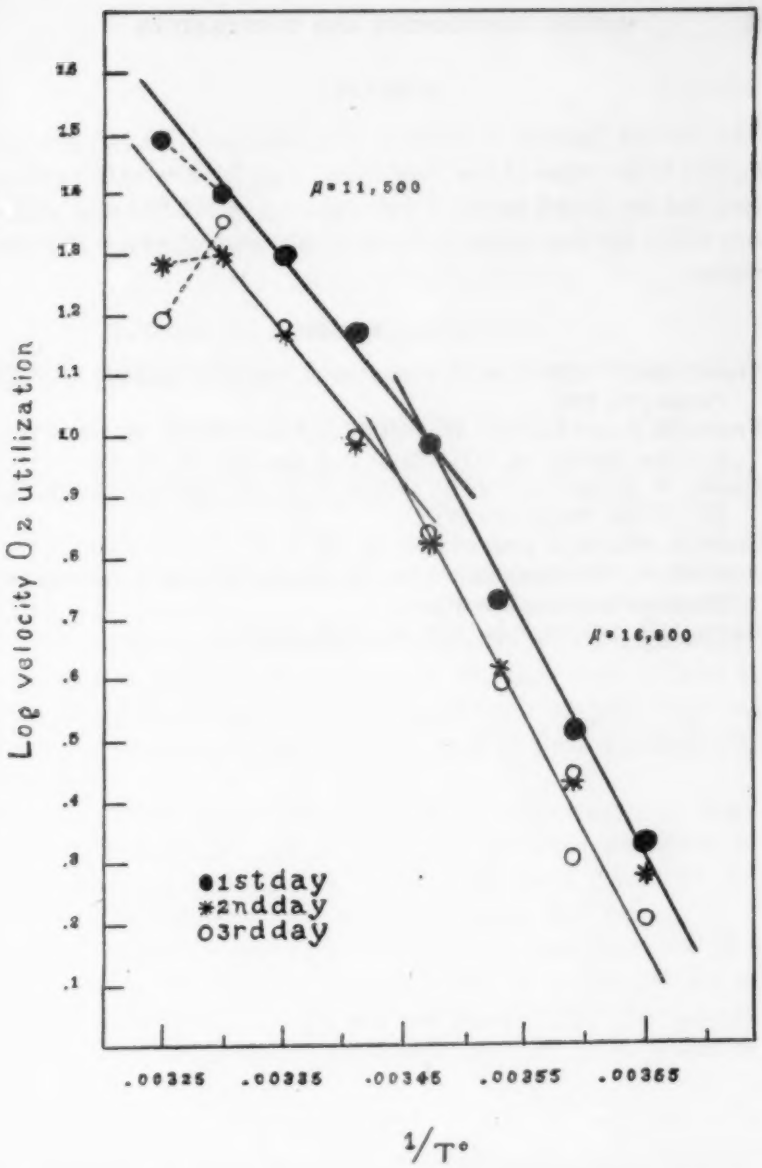

FIG. 1. Oxygen consumption of pupæ of *Drosophila melanogaster* of different ages. Each plotted point is the mean of several experiments. Above 30°C. respiratory activity is irregularly affected, especially in pupæ over 1 day old. Pupæ 4 days old and about to emerge do not give consistent results due undoubtedly to variable body movements of the flies within the pupal cases.

SUMMARY.

The critical thermal increments are calculated for oxygen consumption in the pupæ of the "wild type" fruit fly, *Drosophila melanogaster*, and are found to be of two types: μ = 11,500 and 16,800; above 15°C. the first value is obtained, the second, below this temperature.

BIBLIOGRAPHY.

1. Crozier, W. J., 1924–25, *a*, *J. Gen. Physiol.*, vii, 123; 1924–25, *b*, *J. Gen. Physiol.*, vii, 189.
2. Crozier, W. J., and Federighi, H., 1924–25, *a*, *J. Gen. Physiol.*, vii, 137; 1924–25, *b*, *J. Gen. Physiol.*, vii, 151; 1924–25, *c*, *J. Gen. Physiol.*, vii, 565.
3. Crozier, W. J., and Stier, T. B., 1924–25, *a*, *J. Gen. Physiol.*, vii, 429; 1924–25, *b*, *J. Gen. Physiol.*, vii, 571.
4. Glaser, O., 1924–25, *J. Gen. Physiol.*, vii, 177.
5. Arrhenius, S., 1915, Quantitative laws in biological chemistry, London; 1912, Theories of solutions, New Haven.
6. Bodine, J. H., and Orr, P. R., 1925, *Biol. Bull.*, xlviii, 1.

A SIMPLE MICRO VESSEL WITH ELECTRODE FOR DETERMINING THE HYDROGEN ION CONCENTRATION OF SMALL AMOUNTS OF FLUID.

By JOSEPH HALL BODINE and DAVID E. FINK.

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(Accepted for publication, May 2, 1925.)

Physiological work on insects carried out by the authors necessitated an accurate knowledge of the hydrogen ion concentration of the blood and body fluids of these organisms. Since the blood of many insects is variously pigmented, the colorimetric methods of hydrogen ion determination are necessarily limited in their application. The maximum amounts of fluid available from a single organism are also in many cases considerably less than 1 cc. There is, therefore, need for an electrometric method, with suitable vessel and electrode, for dealing with the small amounts of fluid obtained from such animals.

Since the various precautions necessary for the satisfactory working of electrometric methods have been repeatedly pointed out by many observers and are clearly stated in works of Clark (1) and Michaelis (2), it will be unnecessary to enumerate them here.

The micro electrode and vessel described in this paper are of a type embodying the essential ideas and features as pointed out by Walpole (3), Bishop (4), and De Eds and Hanzlik (5). The amount of fluid required for an estimation is approximately 0.015 to 0.02 cc.

Description.

The vessel is made from Pyrex glass tubing of 12 mm. bore and 14 mm. outside diameter. One end of the tube is drawn out into a capillary tube (1.5 cm. long) which serves for the introduction and removal of the fluid to be estimated (Fig. 1, *K*). The filling of the apparatus depends primarily on the capillarity of this tube. The length of the

entire vessel is about 4 cm. In the side of the vessel, close to the top of the capillary tube and directly opposite the platinum electrode, is fused an arm (1 cm. long) for the introduction of hydrogen (Fig. 1, *N*). Into the large end of the vessel is fitted a one-hole rubber stopper through which a capillary tube, containing the platinum electrode, passes (Fig. 1, *G*). A small amount of vaseline around this capillary tube seals the vessel against escape of hydrogen at this point. The vessel is rigidly held, by a tight fitting rubber stopper, in the center of the tube of a microscope modified as shown in Fig. 1, *H*. In making the microscopic support for the vessel, approximately two-thirds of the brass tube is sawed away (Fig. 1, *F*) leaving intact the rack and pinion coarse and fine adjustments by means of which the vessel can be quickly and accurately lowered or raised in making contact with the saturated KCl bridge below (Fig. 1, *E*). The electrode consists of a piece of No. 22 gauge platinum wire, 1 cm. in length, sealed into a capillary tube approximately 9 cm. long. The capillary tube containing the electrode is accurately centered in a small brass collar with de Khotinsky cement, and held in place in the brass screw adjustment in the top of the microscope tube (Fig. 1, *B*, *C*). A milled brass top (Fig. 1, *D*) tightly fitted into the microscope tube at the top carries the screw adjustment (Fig. 1, *C*) of the capillary tube holding the electrode. Contact with the electrode is made by means of mercury. Both vessel and electrode are so adjusted that the platinum point is exactly centered and can be quickly raised or lowered into the top of the capillary tube of the vessel. The electrode and vessel are rigidly held and are not easily jarred or loosened from their supports. In the stage of the microscope, the bridge, a U-tube (Fig. 1, *L*) filled with saturated

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FIG. 1. Photograph actual size. *A* = wire contact with electrode through mercury; *B* = capillary glass tube holding electrode; *C* = screw for raising or lowering capillary tube with electrode; *D* = milled brass plate fitting in microscope tube and carrying *C*; *E* = coarse adjustment of microscope; *F* = microscope tube; *G* = rubber stopper in top of vessel; *H* = collar from end of microscope tube with perforated rubber stopper within holding vessel; *I* = vessel; *J* = platinum electrode; *K* = capillary tube for fluid tested; *L* = U-tube of saturated KCl bridge; *M* = rubber stopper holding U-tube in center of stage of microscope; *N* = arm fused on vessel for hydrogen intake.

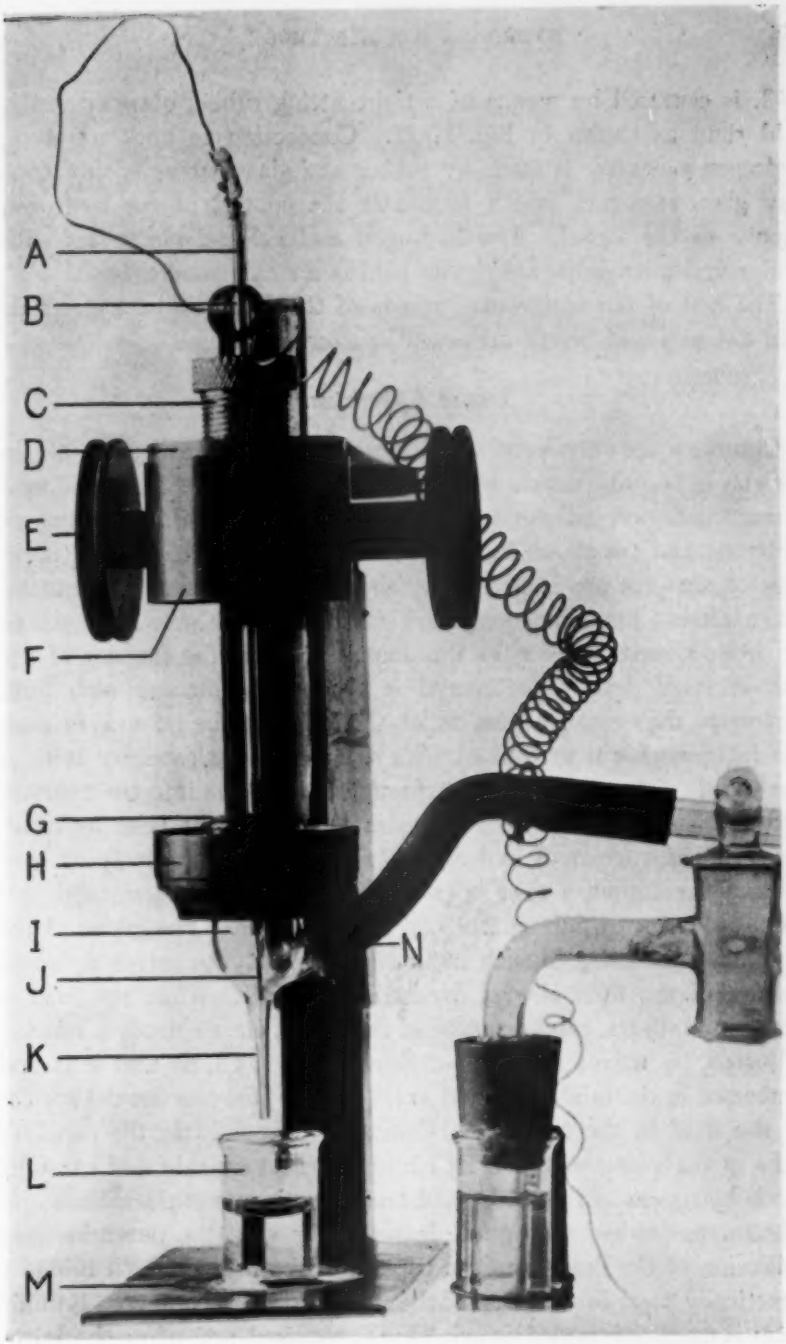

FIG. 1.

KCl, is centered by means of a tight fitting rubber stopper and is held rigid as shown in Fig. 1, *M*. Connection to an electrolytic hydrogen generator is made by rubber and glass tubing with a two-way glass stop-cock which facilitates the control of the hydrogen supply to the vessel. The hydrogen and calomel electrodes with bridge and microscope are rigidly held in a small wooden base.

The rest of the apparatus consists of the usual calomel electrode and a Leeds and Northrup potentiometer outfit.

Use of Apparatus.

Inasmuch as only very small amounts of fluid are available in insects, it is quite impossible to satisfactorily collect the blood in a syringe under oil without severely injuring the animal. The present electrode and vessel are so designed that such procedures in collection of samples are unnecessary, since the fluid examined can be taken almost instantaneously and without appreciable exposure to air from a small incision in the animal or from the interior of the animal itself. After the vessel is thoroughly flushed with pure hydrogen, the organism from which the fluid is to be taken is incised, the incision placed in contact with the tip of the capillary tube of the vessel or the tip introduced through the incision into the animal's body, when the blood is quickly drawn up into the tube by capillarity. The current of hydrogen is shut off simultaneously on contact of the capillary tube with the fluid of the organism and this aids capillarity in pulling the fluid into the tube. The orifice of the capillary tube being of such small diameter, only an extremely small surface of the fluid is ever exposed to the air. After the fluid is drawn up into the capillary tube of the vessel, the electrode is quickly adjusted, by means of the screw head (Fig. 1, *C*), so that it is first immersed in the fluid, and then so that its tip just touches the surface of the fluid in the capillary. Contact of the fluid in the capillary tube of the vessel with the KCl bridge is next quickly and carefully made by means of the coarse and fine adjustments of the microscope. Measurements are then made immediately with the potentiometer. Diffusion of the fluid from the capillary tube into the KCl bridge is practically negligible because of the small surface of contact. Equilibrium is quickly attained; no shaking is required and after the initial

contact of the fluid in the capillary tube with that of the bridge, the capillary tube can be quickly removed from the KCl.

The vessel is easily and quickly cleaned by flushing with hydrogen and repeated washings with distilled water. When dealing with the

TABLE I.

pH Values from Insect Blood and Buffer Solutions by Micro Electrode and Vessel.

Fluid.	pH by micro electrode and vessel.
Buffer solution (5.65).	5.65, 5.67, 5.69, 5.65, 5.65 — average 5.662
Grasshopper blood.	
Melanoplus differentialis, ♂—nymph.	7.15, 7.12, 7.13 — average 7.126
" " ♂—adult.	7.20, 7.20, 7.20 — average 7.20
" " ♀ "	7.25, 7.25, 7.25 — average 7.25
Encoptolophus sordidus, ♀ "	6.9, 7.0 — average 6.95
Japanese beetle larvæ.	
Popillia japonica, 3rd instar.	7.30
Potato beetle.	
Leptinotarsa decemlineata, ♀—adult.	6.0

blood of certain insects the protein content is at times rather high and this necessitates using precautions in the care of the electrode since small coatings of protein tend to cover its point. However, it has been found that many readings can be satisfactorily made with the same electrode before this factor becomes of much significance.

It is of course essential in any electrometric method that accurate checks with known buffer solutions be made before and after determinations on blood and similar fluids. When not in use the capillary tube of the vessel is kept filled with distilled water.

Table I shows a number of results obtained with insect blood as well as with standard buffer solutions. Readings can consistently be made with this method to within 0.02 to 0.05 pH and at times even closer. The accuracy of this method, as with all electrometric methods, depends largely upon the technique employed. With electrodes properly platinized and check solutions accurately made no difficulty has been experienced in duplicating results from day to day. Other types of micro electrodes, such as those described by Bishop (4), De Eds and Hanzlik (5), have been used by the authors, but the present method seems to be much superior to these especially in the rapidity and ease with which such minute samples as those obtained from single insects can be drawn into the vessel without significant exposure to air. It has been found possible to take five to six separate determinations of the blood of a single grasshopper without killing the animal. It thus seems that this electrode is especially useful for work with such small amounts of fluid as are found in insects. In a subsequent paper, results obtained with this method for different insects will be presented.

SUMMARY.

A convenient and accurate method is described for the electrometric determination of the hydrogen ion concentration of small amounts of fluid (0.015 to 0.020 cc.). The suitability of such a method for work on insect blood and body fluids is particularly pointed out.

BIBLIOGRAPHY.

1. Clark, W. M., 1920, The determination of hydrogen ions, Baltimore.
2. Michaelis, L., 1922, Die Wasserstoffionenkonzentration, Berlin.
3. Walpole, G. S., 1913, *Biochem. J.*, vii, 410; 1914, *Biochem. J.*, viii, 131.
4. Bishop, G. H., 1923–24, *J. Biol. Chem.*, lviii, 543.
5. De Eds, F., and Hanzlik, P. J., 1924, *J. Biol. Chem.*, lx, 355.

STUDIES ON LUMINOUS BACTERIA.

II. The Influence of Temperature on the Intensity of the Light of Luminous Bacteria.*

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The fact that temperature plays a large part in the production of light by luminous bacteria has been noted by the majority of observers who have identified new forms since Pflüger described the first form in 1875. In practically all of these descriptions there have appeared the temperature limits at which the bacteria might live, and side by side with these are given the temperature limits for the production of the light. However, nothing has been recorded of the relation which exists between the temperature and the brightness of the light, the former reports being confined entirely to the limits at which the process might be carried on. The purpose of this paper is to show the relation existing between the temperature and the intensity of the light of luminous bacteria.

The minimum temperature for light production in the bacteria is, as a rule, very low (in some cases about −20°C.), due perhaps to the fact that the organisms are of such minute size that the substances within them are contained in capillary spaces, a condition tending to lower the freezing point. Whereas a few degrees rise in temperature above that of the optimum for the production of light rapidly brings a complete loss of the photogenic function and the death of the cell, the temperature of liquid air can be tolerated by the bacteria for a period of several hours (Macfadyen), the light

* This is the second of a series of papers which the writer intends to publish on the reactions of luminous bacteria to varying conditions of environment. The first paper was published in collaboration with Dr. E. N. Harvey in *J. Gen. Physiol.*, 1923–24, vi, 13.

returning when the bacteria are again brought to ordinary temperatures. Since the literature on the temperature limits for luminous bacteria is too extensive to be incorporated in a paper of this nature, the reader is referred for a treatment to Harvey's book, "The nature of animal light."

Beijerinck, who has studied many forms of luminous bacteria, finds that the luminescence ceases with death of the organism and that the luminescence is independent of the respiration. He is of the opinion that the luminescence is produced at the moment of the conversion of peptone into living protoplasm. Later observations by B. Fischer, Forster, Lehmann, Tollhausen, and others upon the production of light at or below 0°C., a point too low for growth, hardly seem to agree with this view of the vital nature of luminescence. Still later observers, notably Dubois, and Harvey (*b*), find that in some forms other than bacteria the production of light is due to the oxidation of a protein-like substrate, *luciferin*, in the presence of an enzyme, *luciferase*.

Although these two substances have been found to be the active elements in several forms, yet one of these, luciferin, has not been definitely isolated from the bacteria. In 1920 Gerretsen published a paper on the reaction of luminous bacteria to ultra-violet light. In this paper, aside from his observations on the effect of the radiations, he describes the method of isolation of the enzyme luciferase, and is led to the conclusion that the substance is of the same nature as the luciferase in other forms. However, thus far the presence of the substrate has not been definitely proven. Although luminous bacteria which have been thoroughly ground up and dried will give a faint luminescence when mixed with fire-fly luciferase (Harvey, *c*), there are other substances, such as the non-luminous portions of the fire-fly, which will give the same result. It is highly probable that in the bacterial cell the luciferin is in some very unstable form which prevents its isolation, spontaneous oxidation taking place as soon as it comes in contact with the air. Regardless of the fact that there is no absolute proof for the presence of luciferin in the bacteria, I do not believe that it would be amiss to consider the phenomenon of light production as being due to the presence of both of these substances. In view of the work which has been done on the pro-

duction of light by other forms, it is almost impossible to make an exception of these organisms. Therefore, in this work the fundamental assumption is made that the process is enzymic in nature.

Apparatus and Methods.

The photometer bench designed by Hyde and Cady and used in the Nela Research Laboratories was used as the foundation for the apparatus. Near one end of the bench was mounted a 6–8 volt 21 c.p. auto headlight lamp through which the current could be varied by means of rheostats controlled by the observer. An ammeter was placed in series with the lamp and read by an assistant in an adjoining room.

Next to the lamp was placed a black plush screen of the usual photometric design. Across the opening of the screen were fastened two filters: a neutral gray, 10 per cent Wratten filter, and a mercury green (No. 62) Wratten filter. The purpose of the former was to permit the lamp to be run at a sufficiently high temperature to reduce the color differences of the filament, and yet to bring the intensity of the light down to that of the bacteria. The latter served to match the color of the lamp with that of the light of the bacteria.

In front of the screen was placed a diffusing surface, and in front of that a comparison cube. This comparison cube consisted of two 90° angle prisms cemented together along their hypothenuses with their adjacent sides silvered in bands, in such a manner that a beam of light passing at right angles to the observer's line of vision would be reflected toward him, while a source of light directly in front of him would be transmitted uninterruptedly to him. The two sources of light would then appear as parallel bands of light, a band of transmitted light being bounded above and below by two equally broad bands of reflected light. The comparatively large surface covered by these two bands of light greatly facilitates comparing the intensities of weak lights of the same color.

On the opposite side of the cube from the observer was placed a container for the bacteria. This container consisted of two parts: a small, rectangular glass bottle (inside width 39 mm.) and a surrounding metal water bath. In one side of the water bath a hole had been cut in which a piece of plain glass was mounted, forming a

window. The bottle, containing the bacteria suspended in a balanced salt solution, was placed next to the window so that the light which the bacteria emitted could be observed through it.

A copious supply of air was bubbled through the suspension at all times in order to obviate any possibility of a decrease in the intensity of the light due to insufficient supply of oxygen. Likewise, an even distribution of the temperature in the water bath was insured by passing a stream of air through the water.

A centigrade thermometer, graduated in fifths of degrees, was placed in the bottle containing the bacteria and all of the readings made on it.

The comparison lamp was measured in terms of millilamberts[1] by means of a Macbeth illuminometer. Measurements were made from the side of the cube toward the observer and then plotted as a function of the current through the lamp. The absorption of the screens was also measured at varying temperatures of the lamp and the percentage transmission taken into account when the results were tabulated.

The species of bacteria used in these experiments was *Bacterium phosphorescens*, isolated from fish obtained at the Princeton Fish Market during the fall of 1923 and identified by means of Gorham's chart after Dahlgren. The light of this form is yellow-green in color, the most intense part of the spectrum lying in the neighborhood of 510 $\mu\mu$. For experimental purposes the bacteria were grown on large enamel pie plates, the organisms being scraped from the medium and then suspended in a balanced salt solution of pH of approximately 7.7 and isotonic with sea water.

A typical experiment would be carried out in the following manner: after the bacteria had been scraped from the medium and suspended in the salt solution, they were placed in the bottle which in turn was placed in the water bath and the entire piece mounted on the photometer. The temperature of the water in the bath was lowered

[1] The lambert is the unit of brightness. When a surface, obeying Lambert's cosine law, emits or reflects one lumen per sq. cm. its brightness is said to be one lambert. For the definitions of other physical light units, the reader is referred to the report published by the Illuminating Engineering Society, New York, N. Y., 1922.

by the addition of ice until it had brought the temperature of the suspension to such a point that the intensity of the light could just be read with ease on the comparison cube. This point was approximately 2°C. A comparison would then be made and the observer would call out the temperature to the assistant in the next room who would then read the ammeter and tabulate the data. When the temperature had been lowered to the desired point and a comparison made, some ice would be removed from the bath and the temperature allowed to rise slowly, readings being taken at as frequent intervals as possible during the rise and the results tabulated. As the temperature approached that of the room it was found that the rate of increase was considerably slowed down. To eliminate the error due to keeping the bacteria at one temperature for too long a time, a low flame was placed under the water bath and the water slowly heated, the higher temperatures being obtained by raising the flame. During the course of one experiment, lasting about 2 hours, as many as sixty values might be tabulated.

RESULTS.

In luminous bacteria one finds a happy combination for a study of this nature. Not only are they of interest from the point of view of their light production, but also one finds here an organism in which energy is emitted in an easily measurable form, and, when taken as an index of a vital phenomenon, can be subjected to a great variety of environmental changes, and a study made of the effects of these changes on the rate of reaction.

Both at excessively high and at very low temperatures the light emitted by the organisms appears yellower in color than that around the optimum temperature. Whether there is an actual change in the quality of the light, or whether this is an example of the Purkinje phenomenon, has not been determined as yet, due mainly to the difficulties encountered in attempting a spectroscopic examination of lights of such low intensities. However, Harvey (*d*) has noted a similar change in the color of the light of the fire-fly and of ostracods, but only at high temperatures, and he has come to the conclusion that the change is a real one and not subjective.

In Fig. 1 the actual points determined during the course of one

experiment are shown. Here the intensity of the light is plotted along the ordinate as a function of the temperature, plotted along the abscissa. The form of the curve is similar to that obtained for other processes in which temperature is one of the variables.

It will be noted that in the neighborhood of the maximum there are points which lie at some distance from the smoothed curve. At first this was thought to have a special significance, and as a result several experiments were performed to see if there were any changes

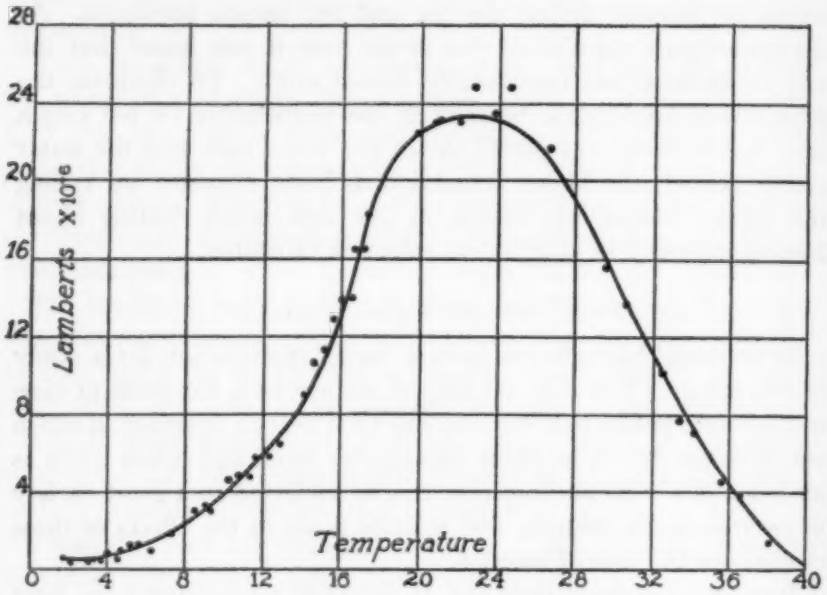

FIG. 1. Curve with experimental points from one emulsion of bacteria showing relation between brightness and temperature.

in the intensity of the light at these points. The temperature in these experiments was maintained at a constant value over a period of 2 to 3 hours; the result of such an experiment is shown in Fig. 2.

It will be seen that the variations which appear in Fig. 1 lie well within the limit of error of setting. To my mind no other explanation of these variations can be given. In calibrating the comparison lamp in terms of photometric units against the current which passes through it, one obtains a curve which rises quite rapidly at

first, but which gradually approaches a line running parallel to the abscissa as the higher intensities are reached. It is easily seen that when one reads the difference in current, which is plotted along the ordinate, the lower values will not give a very great difference in millilamberts, which are plotted along the abscissa, while a corresponding small difference in current at a higher value will give far larger differences in intensity. Thus a difference between 350 and

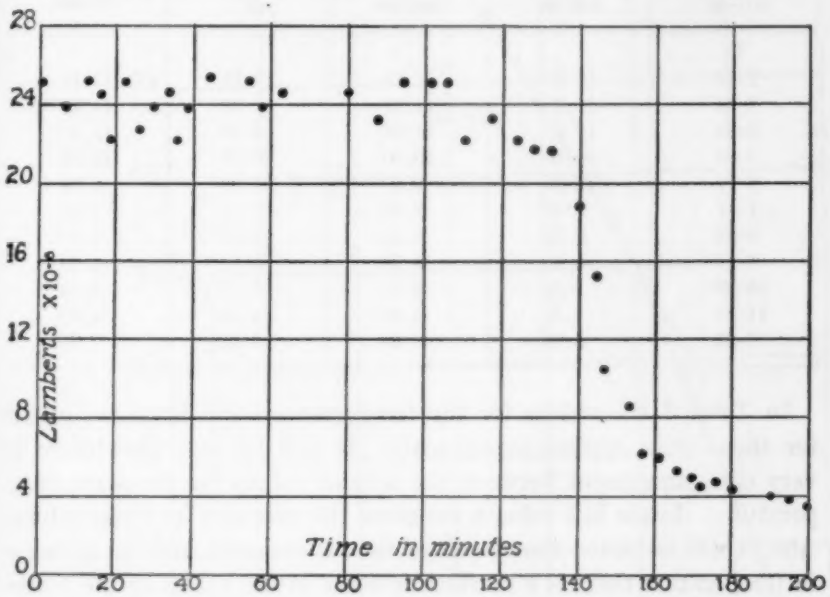

FIG. 2. Curve showing how the brightness of a bacterial emulsion varies with time. Note that the brightness is practically uniform for 2 hours.

352 milliamperes will give a difference in intensity of only 2 millilamberts, while a corresponding difference between 450 and 452 milliamperes will give a difference in intensity of 11 millilamberts.

In common with a good many physiological processes the production of light has a temperature coefficient which does not follow the rule of temperature coefficients laid down by van't Hoff. From the data furnished by this experiment, the Q_{10} for the process was calculated from the van't Hoff formula as modified by Synder:

$$Q_{10} = \left(\frac{k_1}{k_2}\right)^{\frac{10}{t_1 - t_2}}$$

Substitution of the values from the experiment in this equation gives the following list of values for Q_{10}.

TABLE I.

Temperature interval.	Q_{10} June 28.	Q_{10} June 30.	Q_{10} July 3.	Average.
°C.				
2–12	12.72	12.71	12.71	12.71
3–13	11.2	11.35	11.25	11.23
4–14	11.7	11.63	11.69	11.67
5–15	10.80	10.90	10.85	10.85
6–16	10.00	9.97	10.02	9.99
7–17	9.67	9.68	9.65	9.66
8–18	8.52	8.50	8.51	8.51
9–19	6.95	6.94	6.92	6.93
10–20	5.62	5.61	5.65	5.63
11–21	4.72	4.76	4.72	4.73
12–22	3.90	3.89	3.92	3.90

In Table I the values for the temperature coefficients are given for three quite typical experiments. It will be seen that there is very close agreement between the several values for the same temperature. In the last column are given the averages for these values. Also it will be noted that in all of the experiments with an increase in temperature there is a marked decrease in the values of the coefficients, similar to that found in other physiological processes. That this is not the case in the great majority of ordinary chemical reactions is well known, for in these the temperature is more nearly a constant equal to about 2.5. If we assume that the intensity of the light depends upon the reaction velocity in the luciferin-luciferase system, then the discrepancy in the temperature coefficients must be explained. It is possible, indeed probable, that some factor other than the reaction velocity determines the intensity of the light, and that the observed curve is the resultant of these two reactions.

The use of the critical thermal increments, the μ of Arrhenius,

for biological reactions in which the velocity of a reaction is a function of the temperature has been discussed at some length by Crozier.

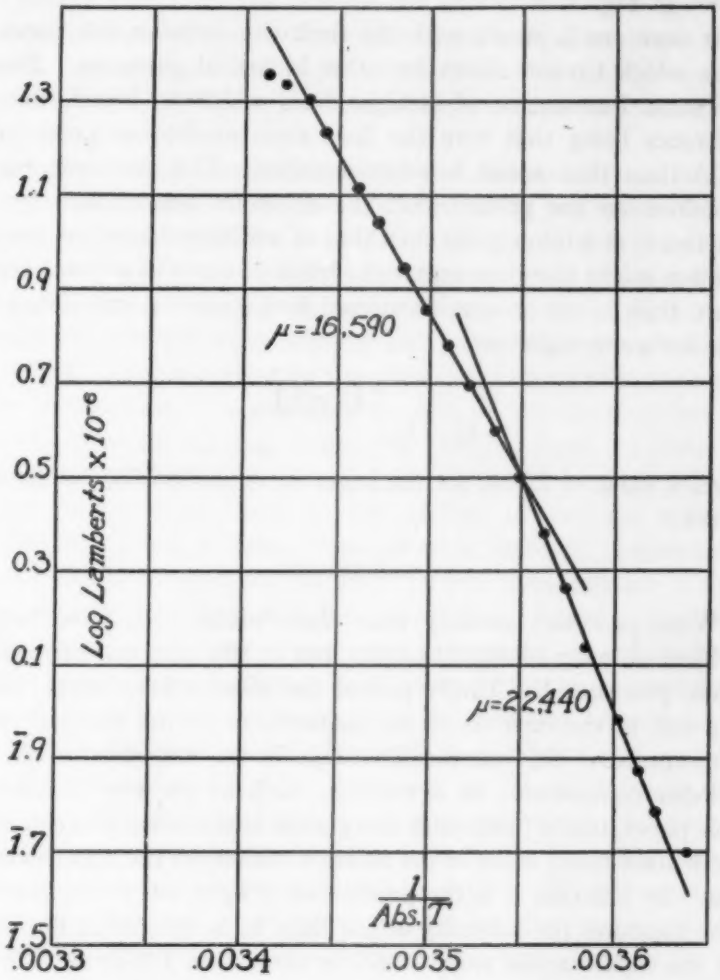

FIG. 3. Data of Fig. 1 plotted as log brightness against the reciprocal of the absolute temperature.

This author is of the opinion that by using the thermal increment rather than the temperature coefficient reactions may be grouped into classes according to the values which they give. In view of this

work it was thought advisable to plot the results in the manner which he suggests. The curve resulting from such a procedure is shown in Fig. 3.

At once one is struck with the similarity between this curve and some which Crozier shows for other biological processes. There is the same intersection of straight lines which he found, the only difference being that here the lines cross at a lower point (about 8°C.) than that found in other reactions. This, however, may be explained on the ground that the optimum temperature for this reaction is at a lower point than that of ordinary biological reactions and one might therefore expect the break to occur at a lower temperature than in the processes analyzed by Crozier. μ calculated from the Arrhenius equation

$$K_2 = K_1 e^{\frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$$

gives a value of 22,440 for the lower curve and 16,590 for the upper curve.

DISCUSSION.

What processes actually take place within cells have been the subject of many biological papers, but at this time our knowledge of these processes has barely passed the observational stage, and we are still in the dark as to the sequence of events and the relative importance of the intermediate steps in the formation of an intercellular compound. In a reaction, such as the one considered in this paper, one is justified in using some outstanding and characteristic feature as an index of the reaction velocity of the underlying process. In this case it is the production of light, but to say that when one measures the intensity of the light he is measuring the velocity of the *light reaction only*, would be erroneous, I believe. Although the production of light by living bacteria is in no way connected with their respiration, it is closely connected with their metabolic processes and ends with their death, so that changes which affect their metabolic activities would affect, of necessity, the production of light. Thus we obtain, I believe, a curve which is not that of the simple light reaction itself, but rather the resultant curve of

several processes which go on simultaneously in the bacterial cell. Indeed this view is substantiated by the form which the curve in Fig. 3 takes. As Crozier has pointed out, this type of curve may be taken as representing a complex reaction, each separate portion of the curve representing control by a separate reaction which is going on and which will influence the rate of the principle activity, that of the production of light.

As a general rule chemical reactions are increased by a rise in temperature in a manner that has been shown by van't Hoff to be equal to two- or threefold for every 10° interval. If such reactions are put in the form of a curve, they will at first slowly rise and then with increasing steepness rapidly become nearly vertical. However, in physiological problems this effect of heat holds only up to a certain point, which varies according to the conditions; up to this point an increase in temperature accelerates the rate of the reaction, but a further rise slows the reaction down, thus bringing about the state of "optimum temperature."

Bayliss, in his monograph on the nature of enzyme action, holds that this effect of heat brings about a state of coagulation of the colloidal particles in the enzyme. In such a coagulation, if the increase in temperature is not too rapid, there occurs a gradual clumping together of the particles and a lessening of the surface over which they are effective. Thus there would be a tendency for the temperature-velocity curve of a physiological phenomenon to rise less steeply and the van't Hoff rule to be abandoned.

From a comparison with other data cited by Crozier (Crozier, *b*) one may consider that the reaction, or reactions, which this curve (Fig. 3) represents are mainly oxidative in nature. For other forms this same conclusion has been reached in an entirely different manner (Harvey, *e*). However, there is apparently another factor which enters into a consideration of the phenomenon, for the values for μ which are obtained point to a process of hydrolysis which goes on at the same time as the process of oxidation. It may be that this process of hydrolysis is accomplished by some enzyme in the bacterial cell which is not directly responsible for the production of light, but which controls some other process linked up very closely with the general metabolism of the cell and which at low temperatures is

slower than that of the luciferin-luciferase reaction, hence acts as a limiting factor in the production of light.

SUMMARY.

1. A method has been described whereby the intensity of the light of luminous bacteria may be measured in a quantitative manner.

2. It is pointed out that the temperature coefficients for light intensity do not follow the van't Hoff rule, but are higher and vary with each 10° temperature interval.

3. From a comparison with other data it is found that the process is not a simple one, but that the observed curve is the resultant of several reactions which proceed simultaneously.

4. The discrepancies in the temperature coefficients in the neighborhood of the "optimum temperature" may be due to a process of coagulation of the colloidal particles of the enzyme. This coagulation will tend to cause a deviation of the curve away from that normal for chemical reactions.

I wish to express my sincere thanks to Professor E. Newton Harvey, of Princeton University, whose interest in the work has made its completion possible. I also wish to express my deep obligation to Dr. W. E. Forsythe, Director of the Nela Research Laboratories, and to the members of his staff, especially Dr. A. G. Worthing and Dr. E. Q. Adams. Dr. Forsythe was kind enough to place a fellowship in the Nela Research Laboratories at my disposal during the summer months of 1924, and it was there that the major part of this work was done.

BIBLIOGRAPHY.

Bayliss, W. M., The nature of enzyme action, London, 4th edition, revised and enlarged, 1919.

Beijerinck, M. W., *Arch. néerl. sc. exactes*, 1891, xxiv, 369.

Crozier, W. J., (*a*) *J. Gen. Physiol.*, 1924–25, vii, 123. (*b*) *J. Gen. Physiol.* 1924–25, vii, 189.

Dahlgren, U., *J. Franklin Inst.*, 1915, clxxx, 513.

Dubois, R., *Compt. rend. Soc. biol.*, 1887, xxxix, 564.

Fischer, B., *Centr. Bakt.*, 1888, iv, 89.

Forster, J., *Centr. Bakt.*, 1892, xii, 431.

Gerretsen, F. C., *Centr. Bakt., 2. Abt.*, 1920–21, lii, 353.

Harvey, E. N., (*a*) The nature of animal light, Monographs on experimental biology, Philadelphia and London, 1920; (*b*) *Am. J. Physiol.*, 1916–17, xlii, 318; (*c*) *Am. J. Physiol.*, 1916, xli, 449; (*d*) *Am. J. Physiol.*, 1924, lxx, 619; (*e*) *Am. J. Physiol.*, 1920, li, 580.

Hyde, E. P., and Cady, F. E., *Elec. Rev. and West. Electr.*, 1912, lxi, 943.

Lehmann, K. B., *Sitzungsber. phys.-med. Ges. zu Würzburg*, 1890, 42.

Macfadyen, A., *Proc. Roy. Soc. London*, 1903, lxxi, 76.

Pflüger, E., *Arch. ges. Physiol.*, 1875, xi, 222.

Snyder, C. D., *Univ. Calif. Pub., Physiol.*, 1905, ii, 535.

Tollhausen, P., Untersuchungen über *Bacterium phosphorescens*, Dissertation, Würzburg, 1889.

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